Pharmacogenomics and Pharmacokinetics of Antiretroviral Drugs and their Associations with Metabolic Complications in HIV-Infected Black South Africans

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Thesis Presented for the Degree of

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DECLARATION

I, Phumla Zuleika Sinxadi, do hereby declare that this thesis includes four journal manuscripts. Three (Chapters 3-5) of the four manuscripts included in this thesis have been published in international peer reviewed journals. The last manuscript (Chapter 6) has also been accepted for publication in an international peer-reviewed journal. The contents of each of these manuscripts remains unchanged from that which has been published or submitted for publication. The manuscripts are listed below, with a description of my contribution and the contribution of each author to the study.

Chapter 3

Sinxadi PZ, McIlleron HM, Dave JA, Smith PJ, Levitt NS, Maartens G. Association of lopinavir concentrations with plasma lipid or glucose concentrations in HIV-infected South Africans: a cross sectional study. *AIDS Res Ther*. 2012 Oct 26; 9(1): 32.

Phumla Sinxadi was the lead investigator on this pharmacokinetics study. She designed the study. She was involved with drawing and processing some of the blood samples. She was responsible for the data entry, analysis, data merging (pharmacokinetics and clinical data given Joel Dave), data interpretation, and drafted the manuscript, which was reviewed critically by all authors.

Helen McIlleron worked on the study design, data interpretation, and critically reviewed the manuscript. Peter Smith performed the analysis of the pharmacokinetic samples and helped to draft the manuscript. Joel Dave worked on the study design, acquisition of clinical data, and critically reviewed the manuscript. Naomi Levitt worked on the study design, acquisition of clinical data and critically reviewed the manuscript. Gary Maartens helped design the study, supervised the study, the data analysis, data interpretation and the writing of the manuscript.

Chapter 4

Sinxadi PZ, Dave JA, Samuels DC, Heckmann JM, Maartens G, Levitt NS, Wester CW, Haas DW, Hulgan T. Mitochondrial genomics and antiretroviral therapy-associated metabolic complications in HIV-infected Black South Africans: a pilot study. *AIDS Res Hum Retroviruses*. 2013 Jul; 29(7): 1031-9.

Phumla Sinxadi was the lead investigator on this pharmacogenetics study. She designed the study. She was involved with drawing and processing some of the blood samples. She did the DNA extraction and shipping to Vanderbilt Genomics Core. She was responsible for the data cleaning, data merging (mitochondrial haplogrouping data from David Samuels, and the clinical data from Joel Dave and Jeanine Heckmann). She performed the data analyses with assistance from Lana Olson. She wrote the manuscript, which was reviewed critically by all authors.

Joel Dave worked on the study design, acquisition of clinical data, and critically reviewed the manuscript. David Samuels worked on the haplogrouping, data interpretation and reviewed the manuscript. Jeanine Heckmann performed the neurological assessments of participants, worked on the study design, and critically reviewed the manuscript. C William Wester critically reviewed the manuscript. Gary Maartens worked on the study design, supervised the study and the writing of the manuscript. Naomi Levitt worked on the study design and she critically reviewed the manuscript. David Haas worked on the study design, data interpretation and he critically reviewed the manuscript. Todd Hulgan designed the study, supervised the data analysis, data interpretation and the writing of the manuscript.

Chapter 5

Sinxadi PZ, Leger PD, McIlleron HM, Smith PJ, Dave JA, Levitt NS, Maartens G, Haas DW. Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa. *Br J Clin Pharmacol.* 2015 Jul; 80(1): 146-56.

Phumla Sinxadi was the lead investigator on this pharmacogenetics study. She designed the study. She was involved with drawing and processing some of the blood samples. She did the DNA extraction and shipping to Vanderbilt Genomics Core. Under David Haas's supervision, she and Paul Leger did the genotyping at Vanderbilt. She was responsible for the data entry, data merging (with pharmacokinetics data from Peter Smith, and clinical data given Joel Dave), data analyses, data interpretation, and drafted the manuscript, which was reviewed critically by all authors. Paul Leger worked on the genotyping, data analysis and interpretation and reviewed the manuscript. Helen McIlleron worked on the study design, data interpretation, and critically reviewed the manuscript. Peter Smith performed the analysis of the pharmacokinetic samples and helped to draft the manuscript. Joel Dave worked on the study design, acquisition of clinical data, and critically reviewed the manuscript. Naomi Levitt worked on the study design, acquisition of clinical data and critically reviewed the manuscript. Gary Maartens helped design the study, supervised the study and the writing of the manuscript. David Haas designed the study, supervised the genotyping, the data analysis, data interpretation and the writing of the manuscript.

Chapter 6

Sinxadi PZ, McIlleron HM, Dave JA, Smith PJ, Levitt NS, Haas DW, and Maartens G. Plasma efavirenz concentrations are associated with lipid and glucose concentrations. Medicine (in press)

Phumla Sinxadi was the lead investigator on this pharmacokinetics study. She designed the study. She was involved with drawing and processing some of the blood samples. She was responsible for the data entry, analysis, data merging (pharmacokinetics and clinical data given Joel Dave), data interpretation, and drafted the manuscript, which was reviewed critically by all authors. Helen McIlleron worked on the study design, data interpretation, and critically reviewed the manuscript. Peter Smith performed the analysis of the pharmacokinetic samples and helped to draft the manuscript. Joel Dave worked on the study design, acquisition of clinical data, and critically reviewed the manuscript. Naomi Levitt worked on the study design, acquisition of

clinical data and critically reviewed the manuscript. David Haas supervised the data analysis, interpretation and the writing of the manuscript. Gary Maartens helped design the study, supervised the study, the data analysis, data interpretation and the writing of the manuscript.

I confirm that no part of this thesis has been submitted in the past, or is being, or is to be submitted for a degree at this or any other university. I hereby grant the University of Cape Town free license to reproduce this thesis in whole or part for the purposes of research or teaching.

This thesis is presented for examination in fulfilment of the requirements for the degree of Doctor of Philosophy in Clinical Pharmacology.

Signed,

Signed by candidate

Phumla Zuleika Sinxadi

25 MAY 2016

ABSTRACT

BACKGROUND: Antiretroviral therapy (ART), notably efavirenz and lopinavir, have been associated with metabolic abnormalities known to increase cardiovascular risk. Efavirenz and lopinavir pharmacokinetics demonstrate considerable interindividual variability, which in part, may be explained by host genetic factors. Mitochondrial DNA (mtDNA) variation influences ART related metabolic complications. However, the associations between genetic polymorphisms and pharmacokinetics of antiretroviral drugs, and their associations with metabolic complications, are incompletely understood. We explored associations of mitochondrial DNA (mtDNA) haplogroups and ART related metabolic complications, characterized relationships between genetic polymorphisms and plasma efavirenz concentrations, and investigated associations between plasma efavirenz/lopinavir concentrations and lipid and glucose concentrations in HIV-infected Black South Africans.

METHODS: We collected clinical and laboratory data from HIV infected patients on ART from Cape Town. We sequenced the mitochondrial genome and determined African mtDNA haplogroups. We genotyped 241 polymorphisms in genes potentially relevant to efavirenz metabolism and transport. We measured steady state efavirenz and lopinavir concentrations and used regression analyses to determine associations with metabolic parameters.

RESULTS: In multivariate regression analyses adjusting for age, sex and ART duration African mtDNA subhaplogroup L3e1 (adjusted OR (aOR) 3.16, p=0.03) and exposure to lopinavir/ritonavir (aOR 2.98, p=0.05) were independently associated with hypertriglyceridemia. There were no significant associations between mtDNA haplogroups and cholesterol, dysglycaemia, hyperlactataemia, lipoatrophy, or peripheral neuropathy.

A model that included composite genotype (*CYP2B6* 516/983/15582) best predicted efavirenz concentrations (β=0.28, p=2.4 x10^{-11}). Among individual *CYP2B6* polymorphisms, 516G→T best

predicted efavirenz concentrations (β =0.22, p=1.27x10^{-6}). There were also associations with 983T→C (β =0.27, p=0.002), and 15582C→T (β=0.11, p=0.04). No other polymorphisms were independently associated with efavirenz concentrations.

In multivariate regression analyses adjusting for age, sex, BMI, and ART duration log_{10} transformed efavirenz concentrations were significantly associated with total cholesterol (β=1.34, p<0.001), LDL cholesterol (β=0.62, p=0.012), HDL cholesterol (β=0.46, p<0.001), triglycerides (β = 0.58, p=0.022), fasting glucose (β=0.60, p=0.017), and 2-hour glucose concentrations (β=1.14, p=0.010).

We found no associations between lopinavir concentrations and metabolic parameters.

CONCLUSIONS: Our findings improve the understanding of genetic determinants of efavirenz plasma exposure in an African population, and provide new insights into host factors associated with ART related metabolic complications.

ACKNOWLEDGEMENTS

The cross-sectional study was funded by the World Diabetes Foundation and the South African Department of Health. The pharmacokinetic analysis was funded by the SAMRC Self-Initiated Research Grant awarded to Professor Gary Maartens. The pharmacogenomics work was supported in part by grants from the National Center for Advancing Translational Sciences, and National Institute of Allergy and Infectious Diseases grants of the National Institutes of Health (AI-077505, AI-054999, K23AI073141, P30AI 060354, UL1 TR000445, UM1 AI068634, UM1 AI068636, UM1 AI106701) and the National Research Foundation of South Africa (90729).

Phumla Sinxadi was supported by the Discovery Foundation, Wellcome Trust Clinical Infectious Diseases Research Initiative Fellowship, South African Medical Research Council (SAMRC) Specialist Training Fellowship and the National Health Scholar Program. She attended a manuscript-writing workshop supported by the South African Tuberculosis and AIDS Training (SATBAT) program (National Institute of Health/Fogarty International Center 1U2RTW007370/05).

I thank Professor Maartens and Professor David Haas, for their patience as they guided me through this journey. Their constant support and encouragement has kept me going even when challenges came our way. I'm grateful for their wise advice along the way, their guidance on manuscript writing, and that I always knew I could count on both of them for the fastest, most detailed critical review. I thank Associate Professor Todd Hulgan for his patient and thorough supervision on the mitochondrial work. I thank Associate Professor Helen McIlleron for her invaluable input on the PK studies.

I thank the patients who volunteered for the studies. I thank the study coordinator, Carmen Delport, and her field team, whose dedication has contributed to the success of these studies. I thank the pharmacology laboratory team for handling and processing pharmacokinetic samples. I

thank the staff at the Vanderbilt Genomics Core laboratory especially Danielle Richardson and Paul Leger for showing the ropes in the laboratory.

I thank all colleagues who participated in the PK-PD studies (Joel Dave, Dinky Levitt, and Peter Smith), and the pharmacogenomics studies (Paul Leger, Jeannine Heckman, David Samuels and Bill Wester). I thank the Ritchie Lab and classmates at Vanderbilt Human Genetics Department for the warm welcome and support when I was miles and miles away from home. I thank the colleagues in the Division of Clinical Pharmacology for their support.

I thank my friends and my big family, for constant support and encouragement. I'd list all of you, but I would run of pages. I especially want to thank my sister, Mimi, who always believed that I should be able to do this with ease. She was wrong! It wasn't easy, but her constant "when are you submitting?" encouraged me to do more, so I could give a better answer the next time she asked. I hope our parents would be proud. Finally, I will be very happy when I can finally tell my granny "ndigqibile ngale mfundo engapheliyo".

Phumla Zuleika Sinxadi.

25 December 2015

CHAPTER 1

Introduction

Chapter 1

Introduction

Context

Sub-Saharan Africa carries the highest global burden of people living with HIV, with an estimated 26 million living in this region.[1,2] Efforts to control the epidemic include up-scaling access to antiretroviral therapy (ART) in low to middle income countries.[1] In 2014, 10.7 million people were accessing ART, with 5/7 people on ART living in Sub-Saharan Africa.[1,2] Improved access to ART has led to an appreciable decline in the incidence of new HIV infections, as well as AIDS related deaths.[1]

South Africa is home to more people living with HIV than any other country.[3] In the 2012 household survey, the HIV prevalence in South Africa was estimated at 12.2% (6.4 million persons), with the prevalence varying greatly by age, race, sex and province. Over 6.2 million black Africans were living with HIV, with the females in their 20s to early 30s being the worst affected.[3] Of the 6.4 million people living with HIV in South Africa, only just over 2 million persons (31.2 %) were exposed to ART.[3]

Standard combination antiretroviral therapy (ART) regimens include two nucleoside reverse transcriptase inhibitors (NRTIs) with a non-NRTI or a ritonavir-boosted protease inhibitor.[4] With the improved access to ART and subsequent decline in mortality, HIV-infected patients are living longer and diseases associated with aging, such as cardiovascular events, are emerging as major concerns for long term HIV management.[5] In addition, exposure to ART has been associated with metabolic adverse effects such as dyslipidaemia, insulin resistance, dysglycaemia, central fat accumulation, peripheral fat loss (lipoatrophy), and peripheral neuropathy.[6,7] Of note, the

association between metabolic adverse events is not usually a class effect, but more related to individual drug exposures.[8]

Some of these metabolic adverse events are thought to be dose-related, as evidenced by lower cumulative incidence when doses are reduced. However, data linking the metabolic adverse events and drug concentrations is limited and sometimes conflicting. For example, our study group found no association between stavudine plasma concentrations and lipoatrophy.[9] In contrast, ter Hosftede et al found such positive association between stavudine and lipoatrophy.[9,10] Pereira et al found a positive correlation between efavirenz plasma concentrations and high-density lipoprotein (HDL) cholesterol,[11] whereas two other studies found no associations.[12,13] Two studies reported a positive correlation between higher lopinavir plasma concentrations and triglycerides but not cholesterol,[14] or both triglycerides and cholesterol concentrations.[15] A few more studies have reported no associations between lopinavir or ritonavir plasma and cholesterol or triglycerides.[13,16-20] These studies are usually small and often not randomized. Therefore, more studies are needed to elucidate the pathogenesis of these ART related metabolic complications. More studies on the relationships between efavirenz and lopinavir drug concentrations and the lipid and glucose concentrations especially relevant in resource-limited settings, where they still form key components of the 1st (efavirenz) and 2nd (lopinavir) line regimens.

There is marked interindividual variability in plasma concentrations of efavirenz and lopinavir, which is partly due to variation in genes encoding metabolism and transport.[21] Efavirenz is metabolized primarily by cytochrome P450 isoenzyme 2B6 (CYP2B6) with minor contributions from CYP2A6 and CYP3A4/5, and direct *N*-glucoronidation from UGT2B7.[22] To-date at least three loss-of-function *CYP2B6* genetic polymorphisms [516G→T (rs3745274)[23-27], 983T→C (rs283992499),[26,28-30] and more recently, 15582C→T (rs4803419)[26]] have been consistently been associated with increased plasma plasma efavirenz concentrations. The marked variability in efavirenz concentrations can be partly explained by varying frequencies in these genetic

polymorphisms. For example, our group has shown that *CYP2B6* 516T is about 5 times more prevalent in southern Africans than in Caucasians.[31] The effect of *CYP2B6* 983T→C on efavirenz concentrations (per allele) is greater than that of *CYP2B6* 516G→T, but its frequency is far less and appears to found only with African ancestry.[21,26] The effect of *CYP2B6* 15582C→T is far less than that of *CYP2B6* 516G→T, but the frequency is high with European and Asian ancestry.[26] These three polymorphisms stratify patients into ten plasma trough concentration subgroups across an approximately tenfold range of medians, with the top three classified as slow metabolizers.[26] Therefore, in patients of African ancestry, where the burden of HIV infection largely lies, a high proportion of patients will belong to the slow metabolizer subgroup, with resultant higher efavirenz concentrations, which in turn, may be associated with efavirenz related metabolic complications.

On the other hand, lopinavir is metabolized primarily by hepatic cytochrome P450 (CYP) 3A isoforms and is a substrate for p-glycoprotein (P-gp), multidrug resistance-associated protein 2 (MRP2), and solute carrier organic anion transporter family, member 1B1 (SLCO1B1).[32] Limited studies have found no association between lopinavir plasma concentrations and polymorphisms in *ABCB1, CYP3A5, CYP2B6* and *CYP2D6* genes.[33] Recent studies have reported "gain of function" (rs11045819) and "loss of function" (rs4149056) variants in *SLCO1B1* gene, which may lead to low and high plasma concentrations of lopinavir, respectively.[32,33] It has been shown that polymorphisms in the *PXR* and *C*AR genes in sub-Saharan African adults can alter the induction of CYP3A4 and CYP2B6 promoter activity thus contributing to the variable nature of lopinavir and efavirenz drug interactions, respectively.[34]

Several metabolic abnormalities are due to mitochondrial toxicity from NRTI inhibition of mitochondrial DNA (mtDNA) polymerase–γ, an essential enzyme for mtDNA replication.[6] Manifestations of mitochondrial toxicity include distal sensory polyneuropathy (DSP), hyperlactataemia, lipoatrophy and insulin resistance.[35,36] Our collaborators at Vanderbilt have

identified associations between mtDNA haplogroups and NRTI related complications. Associations between mtDNA haplogroups and other metabolic complications, including lipoatrophy and dyslipidaemia have been reported.[37,38] In European Americans mitochondrial haplogroup T independently increased the risk of DSP.[39] In African Americans, conflicting results were found regarding the association between African mtDNA sub-haplogroup L1c and DSP.[40-42]

We hypothesized that antiretroviral pharmacokinetics and pharmacogenomics predict metabolic complications (including DSP) of antiretroviral therapy in a well-characterized cohort of HIV-infected black South Africans. The key research questions addressed in this thesis are listed below.

Key research questions

The key research questions addressed in this thesis are:

1. Are efavirenz concentrations associated with lipids and glucose concentrations?
2. Are lopinavir concentrations associated with lipids and glucose concentrations?
3. Is the interindividual variability in plasma efavirenz concentrations predicted by genetic polymorphisms in *CYP2B6*, alone or in combination with polymorphisms in additional relevant genes (*CYP2A6, ABCB1, CYP3A4, CYP3A5* and *PXR)*?
4. Are the African mitochondrial DNA (mtDNA) haplogroups associated with toxicity due to stavudine or zidovudine (DSP, lipoatrophy, hypertriglyceridaemia, hyperlactataemia, and dysglycaemia)?

Setting

Antiretroviral treatment program in Cape Town

South Africa is home to the highest number of people living with HIV infection.[1] Prior to the antiretroviral roll-out by the National Department of Health in late 2003, access to the antiretroviral drugs limited to places where ART clinics were set up by not-for-profit organizations like the Doctors Without Borders, enrollment into clinical trials for mother-to-child prevention programs or patients paid for the antiretroviral drugs through private medical practice.

Since 2004, access to ART has improved dramatically in South Africa.[1] The treatment guidelines prioritized access to people who met the eligibility criterion of a CD4+ count threshold of <200 cells/μl or clinical criteria according to World Health Organization (WHO) stage 3 or 4. In adults, the national treatment guidelines recommended a 1st line regimen that included a weight based stavudine dosing (40 mg if weight above 60 kg, 30 mg if weight below 60kg), fixed dose lamivudine (150 mg 12 hourly) and efavirenz (600 mg at night) or nevirapine (200 mg daily for the 2 weeks, increasing to 200 mg twice daily). For 2nd line regimen, zidovudine (300 mg 12 hourly), didanosine (400mg once daily, 250 mg once daily if weight below 60 kg) and ritonavir-boosted lopinavir (400/100 mg 12 hourly).

Although the WHO ART guidelines for resource-limited settings urged countries "to begin planning to move away from stavudine-containing regimens" in 2006, stavudine continued to be widely used in standardized first-line regimens in low- and middle-income countries as it had a low acquisition cost, was available in fixed dose combination formulations and did not require laboratory monitoring for toxicity.[9] WHO then recommended reduced doses of stavudine following the findings of a systematic review that lower doses caused less toxicity without reducing efficacy.[43] Programmatic delays allowed continuation of high dose stavudine in a few patients who were enrolled in the cross-sectional study described below.

ART access program in South Africa has designated ART clinics that are located in primary care clinics, secondary level hospitals or tertiary care clinic. We recruited from two Cape Town ART clinics. The first clinic was based in Groote Schuur Hospital, a referral centre for ART access for patients diagnosed while in this hospital or patients referred from primary care clinics. The second clinic was based in Cross Roads Day Hospital, a primary care centre located in a township.

Main prevalence study

The Metabolic complications of HAART (McHAART) study is a cross sectional study, whose aim was to determine the prevalence of metabolic complications of ART. Ambulatory HIV-infected patients on the South African National Department of Health 1st line ART regimen (stavudine or zidovudine, each with lamivudine, PLUS efavirenz or nevirapine), 2nd line ART regimen (zidovudine, didanosine, PLUS ritonavir-boosted lopinavir) and those not yet on ART (ART-naïve) were recruited by convenient sampling consecutively from a list at two Cape Town ART clinics between 2007 and 2009. Patients were excluded if they had a history of diabetes mellitus or impaired glucose tolerance (IGT), had been on ART for less than 6 months, had an active acute opportunistic infection, had severe diarrhea (>6 stools/d), had tuberculosis within 1 month of starting treatment, had received glucocorticoid therapy within the past 6 months, or were pregnant or known to have renal failure. The Research Ethics Committee of the Faculty of Health Sciences of the University of Cape Town approved the study. Before participating in the study, procedures and risks were explained to the subjects, who gave written informed consent to participate in the study. Additional consent was obtained for storage of genetic samples and linkage with clinical data.

In brief, after screening and enrolment, participants were given an appointment date, instructed to fast the night before the appointment, and record the last dose of ingesting, where applicable. On the study day, participants underwent an oral glucose tolerance test (OGTT). Blood was drawn at 0 and 120 minutes after ingesting 75 g of glucose in 250 mL of water, and kept on ice until

centrifuged within 4 hours. Plasma for antiretroviral quantification was collected into 4mL lithium heparin tubes, kept on ice until centrifuged within 4 hours, and was aliquotted and promptly frozen at −20°C, then stored at -70°C until analysis at the end of recruitment in 2009. Plasma for antiretroviral drugs, fasting glucose, cholesterol, and triglyceride were quantified using the 0 minute OGTT samples. Finger-prick lactate was measured. Buffy coats were collected from EDTA tubes and stored in the -80 freezer until DNA extraction.

Clinical assessments were done in all participants. A neurologist examined a subset of participants to determine neuropathy status. Some participants underwent dual energy X-ray absorptiometry (DEXA) scans to measure bone density and body fat percentage.

Self reported adherence was determined using a validated standard 4-day adherence questionnaire administered by trained field workers. We reviewed medical records to determine duration on antiretroviral therapy and current CD4+ lymphocyte counts and viral load. Current CD4+ count was regarded as the count measured within 3 months of the study visit.

Standard criteria for clinical and laboratory evaluations were used and described in the first three publications that arose from the cross sectional study.[9,44,45] Preliminary analysis of OGTTs in 339 participants showed 2% had diabetes and 24% had impaired fasting glucose or glucose tolerance.[45] In multivariate analysis, efavirenz emerged as a risk factor for dysglycaemia –a novel finding requiring additional research. The prevalence of DSP was 49% in 598 participants and was significantly associated with stavudine use.[44] We measured plasma stavudine concentrations during the OGTT in 47 participants. We used a population pharmacokinetic approach to estimate the stavudine area under the concentration time curve (AUC) and found no association between stavudine AUC and lipoatrophy, glucose, lactate and triglyceride concentrations.[9]

Departmental collaborations at the University of Cape Town (UCT)

The cross-sectional study was collaboration between three divisions within the Department of Medicine at the University of Cape Town. These are: Division of Endocrinology and Diabetic Medicine, Division of Neurology, and the Division of Clinical Pharmacology. The Division of Endocrinology and Diabetic Medicine conducted the main prevalence study looking at the metabolic complications of ART. The Division of Neurology conducted neurological assessment in selected patients. The Division of Clinical Pharmacology conducted the pharmacokinetics and pharmacogenetics sub-studies reported in this thesis and I (Phumla Zuleika Sinxadi), was the lead investigator on all. Of note, the pharmacology laboratory at the University of Cape Town is one of the AIDS Clinical Trial Group (ACTG) approved site, has world accreditation, and has conducted all the pharmacokinetics analyses reported in the studies included in this thesis.

UCT –Vanderbilt University collaboration

The pharmacogenomics components of this thesis were done in collaboration with Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America. The studies done complied with the Helsinki Declaration and were approved by institutional review boards for each site. Only participants who gave separate consent for genetics storage and analyses were enrolled. Participants or their parents gave written consent or assent as appropriate.

Clinical and laboratory (including pharmacokinetic analyses) data were collected in Cape Town. Genotyping and mitochondrial sequencing was done at Vanderbilt University. This minimal risk study used existing stored samples for DNA and pharmacokinetic analyses and clinical data from the McHAART study. In addition, pooled analyses with a paediatric study of African children on efavirenz based therapy and off tuberculosis therapy were enrolled for the study investigations genetic predictors of plasma efavirenz concentrations. We utilized stored DNA for genotyping and mitochondrial sequencing done at Vanderbilt University.

Our collaborators at Vanderbilt, with Todd Hulgan as the lead investigator, have identified associations between mtDNA haplogroups and ART related complications. My co-supervisor, David Haas, has led seminal that demonstrated that a frequent *CYP2B6* variant (516G→T) predicted decreased plasma efavirenz clearance and increased plasma efavirenz concentrations. Professor Gary Maartens, my main supervisor, has conducted several studies in HIV and TB, and one of the key opinion leaders in the field.

Chronology of the studies

The chronology of the studies constituting this thesis is set out according to the publication date of the papers.

Chapter 3

Sinxadi PZ, McIlleron HM, Dave JA, Smith PJ, Levitt NS, Maartens G. Association of lopinavir concentrations with plasma lipid or glucose concentrations in HIV-infected South Africans: a cross sectional study. *AIDS Res Ther*. 2012 Oct 26; 9(1): 32. doi: 10.1186/1742-6405-9-32. PubMed PMID: 23098156; PubMed Central PMCID: PMC3533776.

The aim was to characterize associations between lopinavir plasma concentrations and the lipids and glucose concentrations.

Chapter 4

Sinxadi PZ, Dave JA, Samuels DC, Heckmann JM, Maartens G, Levitt NS, Wester CW, Haas DW, Hulgan T. Mitochondrial genomics and antiretroviral therapy-associated metabolic complications in HIV-infected Black South Africans: a pilot study. *AIDS Res Hum Retroviruses*. 2013 Jul; 29(7):1031-9. doi: 10.1089/AID.2012.0373. Epub 2013 Mar 15. PubMed PMID: 23428049; PubMed Central PMCID: PMC3685683.

We explored associations between African mitochondrial DNA (mtDNA) haplogroups and ART-associated lipoatrophy, dysglycaemia, hypertriglyceridaemia, and/or distal peripheral neuropathy

in Black HIV- infected South African population.

Chapter 5

Sinxadi PZ, Leger PD, McIlleron HM, Smith PJ, Dave JA, Levitt NS, Maartens G, Haas DW. Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa. *Br J Clin Pharmacol.* 2015 Jul; 80(1):146-56. doi: 10.1111/bcp.12590. Epub 2015 May 28. PubMed PMID: 25611810; PubMed Central PMCID: PMC4500334.

The aim was to determine whether interindividual variability in plasma efavirenz concentrations is predicted by genetic polymorphisms in *CYP2B6*, alone or in combination with polymorphisms in additional relevant genes (*CYP2A6, ABCB1, CYP3A4, CYP3A5* and *PXR)*. We included adults and children who had been enrolled in two observational studies. In the adult study, adults from the public sector antiretroviral therapy (ART) program were enrolled in a cross sectional study to evaluate the associations between plasma efavirenz concentrations and metabolic complications. In the paediatric study, African children on efavirenz-based therapy with or without rifampicin-based antituberculosis therapy were enrolled to evaluate the effect of rifampicin-based antituberculosis therapy on efavirenz concentrations. The present analyses included only data from children who were not receiving rifampicin.

Chapter 6.

Sinxadi PZ, McIlleron HM, Dave JA, Smith PJ, Levitt NS, Haas DW, Maartens G. Plasma efavirenz concentrations are associated with lipid and glucose concentrations. *Medicine* (in press).

The aim was to characterize associations between efavirenz plasma concentrations and the lipids and glucose concentrations.

Outline of the thesis

In **Chapter 2** (Background and literature review) literature review regarding the prevalence of the metabolic complications is presented. The data is divided into two parts, 1) data of the prevalence of ART related metabolic complications in high-income countries, 2) data of the prevalence of ART related metabolic complications in Sub-Saharan Africa, where the majority of the people living with HIV reside. Thereafter, more in-depth literature review on the four topics relevant to the studies presented in this thesis; data linking associations between the metabolic complications and efavirenz and lopinavir plasma concentrations; pharmacogenetics of efavirenz; and lastly, mitochondrial genomics and metabolic complications.

In **Chapter 3** the cross sectional study whose aim was to characterize associations between lopinavir plasma concentrations and lipid and glucose concentrations is reported. This lopinavir pharmacokinetic-pharmacodynamic study is included as a full manuscript with the introduction, methods, results and discussion and conclusions.

In **Chapter 4** a pilot study whose aim was to explore associations between African mitochondrial DNA haplogroups and metabolic complications (dyslipidaemia, dysglycaemia, hyperlactataemia, lipoatrophy and distal peripheral neuropathy) is reported. This mitochondrial genomics study is included as a full manuscript with the introduction, methods, results, discussion and conclusions.

In **Chapter 5** the pooled analyses whose aim was to determine whether interindividual variability in plasma efavirenz concentrations is predicted by genetic polymorphisms in *CYP2B6*, alone or in combination with polymorphisms in additional relevant genes (*CYP2A6, ABCB1, CYP3A4*, *CYP3A5* and *PXR)* is reported. This efavirenz pharmacokinetics-pharmacogenetics study is included as a full manuscript with the introduction, methods, results, discussion and conclusions.

In **Chapter 6** the cross sectional study whose aim was to characterize associations between efavirenz plasma concentrations and lipid and glucose concentrations is reported. In a subset of

participants with available *CYP2B6* genetic polymorphism data, associations between *CYP2B6* polymorphisms and lipids and glucose concentrations is explored. This efavirenz pharmacokinetic-pharmacodynamic-pharmacogenetics study is included as a submitted manuscript with the introduction, methods, results and discussion and conclusions.

In **Chapter 7** the findings of the four studies are summarized and conclusions from across all the studies are presented. The implications of the research for the field in general are discussed and priorities for future ART related complications are identified.

Coherence of the thesis

I (Phumla Zuleika Sinxadi) am the first author on all four papers included in this thesis, and was the lead investigator on all the studies. Professors Gary Maartens and David Haas jointly supervised the studies while I was registered as a student at the University of Cape Town, including when I visited Vanderbilt University as a non-degree seeking student. All four studies include participants recruited from the same cross-sectional study that investigated the prevalence of ART- associated metabolic complications. The body of work from all studies provide data that may improve understanding of the pathogenesis of ART-related complications among populations hardest hit by HIV by looking at genomic predictors of metabolic complications or drug concentrations, and the associations between drug concentrations and these metabolic complications. This may ultimately contribute to “personalized medicine” for the HIV-infected community worldwide.

Bibliography and References Cited

1. UNAIDS. 90-90-90 An ambitious treatment target to help end AIDS epidemic. 2014; http://www.unaids.org/en/resources/documents/2014/90-90-90.

2. UNAIDS. How AIDS changed everything. MGD 6: 15 years, 15 lessons of hope from the AIDS response. 2015; http://www.unaids.org/en/resources/documents/2015/20150714_factsheet.

3. Shisana O RT, Simbayi LC, Zuma K, Jooste S, Zungu N, et al. *South African National HIV Prevalence, Incidence and Behaviour Survey,2012*. Cape Town: HSRC Press;2014.

4. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and prenting HIV infection. 2013; http://www.who.int/hiv/pub/guidelines/arv2013/download/en/.

5. Friis-Moller N, Weber R, Reiss P, et al. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. *AIDS.* 2003;17(8):1179-1193.

6. Kohler JJ, Lewis W. A brief overview of mechanisms of mitochondrial toxicity from NRTIs. *Environmental and molecular mutagenesis.* 2007;48(3-4):166-172.

7. Falutz J. HIV infection, body composition changes and related metabolic complications: contributing factors and evolving management strategies. *Curr Opin Clin Nutr Metab Care.* 2011;14(3):255-260.

8. Hawkins T. Understanding and managing the adverse effects of antiretroviral therapy. *Antiviral Res.* 2010;85(1):201-209.

9. Sinxadi PZ, van der Walt JS, McIlleron HM, et al. Lack of association between stavudine exposure and lipoatrophy, dysglycaemia, hyperlactataemia and hypertriglyceridaemia: a prospective cross sectional study. *AIDS Res Ther.* 2010;7:23.

10. ter Hofstede HJ, Koopmans PP, Burger DM. Stavudine plasma concentrations and lipoatrophy. *J Antimicrob Chemother.* 2008;61(4):933-938.

11. Pereira SA, Branco T, Corte-Real RM, et al. Long-term and concentration-dependent beneficial effect of efavirenz on HDL-cholesterol in HIV-infected patients. *Br J Clin Pharmacol.* 2006;61(5):601-604.

12. Autar RS, Boyd MA, Wit FW, et al. Relationships between drug exposure, changes in metabolic parameters and body fat in HIV-infected patients switched to a nucleoside sparing regimen. *Antivir Ther.* 2007;12(8):1265-1271.

13. Clevenbergh P, Garraffo R, Dellamonica P. Impact of various antiretroviral drugs and their plasma concentrations on plasma lipids in heavily pretreated HIV-infected patients. *HIV Clin Trials.* 2003;4(5):330-336.

14. Gonzalez de Requena D, Blanco F, Garcia-Benayas T, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Correlation between lopinavir plasma levels and lipid abnormalities in patients taking lopinavir/ritonavir. *AIDS patient care and STDs.* 2003;17(9):443-445.

15. Gutierrez F, Padilla S, Navarro A, et al. Lopinavir plasma concentrations and changes in lipid levels during salvage therapy with lopinavir/ritonavir-containing regimens. *J Acquir Immune Defic Syndr.* 2003;33(5):594-600.

16. Leon A, Martinez E, Sarasa M, et al. Impact of steady-state lopinavir plasma levels on plasma lipids and body composition after 24 weeks of lopinavir/ritonavir-containing therapy free of thymidine analogues. *J Antimicrob Chemother.* 2007;60(4):824-830.

17. Torti C, Quiros-Roldan E, Regazzi-Bonora M, et al. Lipid abnormalities in HIV-infected patients are not correlated with lopinavir plasma concentrations. *J Acquir Immune Defic Syndr.* 2004;35(3):324-326.

18. Rhee MS, Hellinger JA, Sheble-Hall S, Cohen CJ, Greenblatt DJ. Relationship between plasma protease inhibitor concentrations and lipid elevations in HIV patients on a double-boosted protease inhibitor regimen (saquinavir/lopinavir/ritonavir). *J Clin Pharmacol.* 2010;50(4):392-400.

19. Bierman WF, van Vonderen MG, Veldkamp AI, et al. The lopinavir/ritonavir-associated rise in lipids is not related to lopinavir or ritonavir plasma concentration. *Antivir Ther.* 2011;16(5):647-655.

20. Ter Hofstede HJ, Koopmans PP, Burger DM, et al. Lopinavir Plasma Concentrations and Serum Lipids in Therapy Naïve HIV-Patients: A Sub Analysis of the FREE Study. *Pharmacology & Pharmacy.* 2012;3:90-96.

21. Aung AK, Haas DW, Hulgan T, Phillips EJ. Pharmacogenomics of antimicrobial agents. *Pharmacogenomics.* 2014;15(15):1903-1930.

22. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther.* 2012;92(4):414-417.

23. Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS.* 2004;18(18):2391-2400.

24. Ribaudo HJ, Liu H, Schwab M, et al. Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. *J Infect Dis.* 2010;202(5):717-722.

25. Haas DW, Smeaton LM, Shafer RW, et al. Pharmacogenetics of long-term responses to antiretroviral regimens containing Efavirenz and/or Nelfinavir: an Adult AIDS Clinical Trials Group Study. *J Infect Dis.* 2005;192(11):1931-1942.

26. Holzinger ER, Grady B, Ritchie MD, et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. *Pharmacogenet Genomics.* 2012;22(12):858-867.

27. Cummins NW, Neuhaus J, Chu H, et al. Investigation of Efavirenz Discontinuation in Multi-ethnic Populations of HIV-positive Individuals by Genetic Analysis. *EBioMedicine.* 2015;2(7):706-712.

28. Wang J, Sonnerborg A, Rane A, et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenet Genomics.* 2006;16(3):191-198.

29. Wyen C, Hendra H, Siccardi M, et al. Cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) polymorphisms are associated with early discontinuation of efavirenz-containing regimens. *J Antimicrob Chemother.* 2011;66(9):2092-2098.

30. Wyen C, Hendra H, Vogel M, et al. Impact of CYP2B6 983T>C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. *J Antimicrob Chemother.* 2008;61(4):914-918.

31. Cohen K, Grant A, Dandara C, et al. Effect of rifampicin-based antitubercular therapy and the cytochrome P450 2B6 516G>T polymorphism on efavirenz concentrations in adults in South Africa. *Antivir Ther.* 2009;14(5):687-695.

32. Lubomirov R, di Iulio J, Fayet A, et al. ADME pharmacogenetics: investigation of the pharmacokinetics of the antiretroviral agent lopinavir coformulated with ritonavir. *Pharmacogenet Genomics.* 2010;20(4):217-230.

33. Kohlrausch FB, de Cassia Estrela R, Barroso PF, Suarez-Kurtz G. The impact of SLCO1B1 polymorphisms on the plasma concentration of lopinavir and ritonavir in HIV-infected men. *Br J Clin Pharmacol.* 2010;69(1):95-98.

34. Svard J, Spiers JP, Mulcahy F, Hennessy M. Nuclear receptor-mediated induction of CYP450 by antiretrovirals: functional consequences of NR1I2 (PXR) polymorphisms and differential prevalence in whites and sub-Saharan Africans. *J Acquir Immune Defic Syndr.* 2010;55(5):536-549.

35. Maagaard A, Kvale D. Mitochondrial toxicity in HIV-infected patients both off and on antiretroviral treatment: a continuum or distinct underlying mechanisms? *J Antimicrob Chemother.* 2009;64(5):901-909.

36. Maagaard A, Kvale D. Long term adverse effects related to nucleoside reverse transcriptase inhibitors: clinical impact of mitochondrial toxicity. *Scand J Infect Dis.* 2009;41(11-12):808-817.

37. Hulgan T, Haubrich R, Riddler SA, et al. European mitochondrial DNA haplogroups and metabolic changes during antiretroviral therapy in AIDS Clinical Trials Group Study A5142. *AIDS.* 2011;25(1):37-47.

38. Hulgan T, Tebas P, Canter JA, et al. Hemochromatosis gene polymorphisms, mitochondrial haplogroups, and peripheral lipoatrophy during antiretroviral therapy. *J Infect Dis.* 2008;197(6):858-866.

39. Canter JA, Haas DW, Kallianpur AR, et al. The mitochondrial pharmacogenomics of haplogroup T: MTND2*LHON4917G and antiretroviral therapy-associated peripheral neuropathy. *Pharmacogenomics J.* 2008;8(1):71-77.

40. Canter JA, Robbins GK, Selph D, et al. African mitochondrial DNA subhaplogroups and peripheral neuropathy during antiretroviral therapy. *J Infect Dis.* 2010;201(11):1703-1707.

41. Hulgan T, Haas DW, Haines JL, et al. Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: an adult AIDS clinical trials group study. *AIDS.* 2005;19(13):1341-1349.

42. Holzinger ER, Hulgan T, Ellis RJ, et al. Mitochondrial DNA variation and HIV-associated sensory neuropathy in CHARTER. *Journal of neurovirology.* 2012;18(6):511-520.

43. WHO. Addendum to the 2006 WHO guidelines on antiretroviral therapy for HIV infection in adults and adolescents. 2006.

44. Maritz J, Benatar M, Dave JA, et al. HIV neuropathy in South Africans: frequency, characteristics, and risk factors. *Muscle Nerve.* 2010;41(5):599-606.

45. Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr.* 2011;57(4):284-289.

CHAPTER 2

Background and literature review

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Sub-Saharan Africa carries the highest global burden of people living with HIV, with an estimated 26 million living in this region.[1,2] Efforts to control the epidemic include up-scaling access to antiretroviral therapy (ART) in low to middle income countries.[1] In 2014, 10.7 million people were accessing ART, with 5/7 people on ART living in Sub-Saharan Africa.[1,2] Improved access to ART has led to an appreciable decline in the incidence of new HIV infections, as well as AIDS related deaths.[1]

South Africa is home to more people living with HIV than any other country.[3] In the 2012 household survey, the HIV prevalence in South Africa was estimated at 12.2% (6.4 million persons), with the prevalence varying greatly by age, race, sex and province. Over 6,2 million black Africans were living with HIV, with the females in their 20s to early 30s being the worst affected.[3] Of the 6,4 million people living with HIV in South Africa, only just over 2 million persons (31.2 %) were exposed to ART.[3]

The UNAIDS 90-90-90 2020 goal is an ambitious treatment target that aims to end an AIDS epidemic. Their goals are to diagnose 90% of people living with HIV by 2020, treat 90% of people living with HIV by 2020, and to achieve 90% of viral suppression in people receiving ART.[1] This ambitious treatment target aims to increase ART coverage by treating early after diagnosis irrespective of CD4+ count, which is projected to increase gains by averting infections, reducing morbidity and mortality. Therefore, people living with HIV will have longer duration of exposure to antiretroviral drugs, and will be susceptible to ART related complications, including metabolic complications, arising from cumulative exposure.

In children aged ≥3 years and adults, the World Health Organization (WHO) recommends combination ART consisting of a nucleoside (nucleotide) reverse transcriptase inhibitor (NRTI)

backbone with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI) for first and second line regimens.[4]

Prevalence of metabolic complications of ART

Studies conducted in Europe, United States and Australia

Antiretroviral drugs and dyslipidaemia

The prevalence of metabolic complications is well studied in high-income countries. In 2003, the Data collection on Adverse events of anti-HIV Drugs (DAD study), a multinational, tri-continental collaboration between established HIV cohorts with over 20000 participants reported that combination ART use was associated with an increased risk of myocardial infarction.[5] The DAD study group also reported that risk factors for cardiovascular disease (myocardial infarction and stroke) were independently associated with PI-containing regimens or NNRTI-containing regimens, or regimens containing both PIs and NNRTIs.[6] The risk of dyslipidaemia was strongly associated with the combined use of PIs and NNRTIs, suggesting additive effects.[6] The DAD study group published several other studies including at study looking at individual drugs,[7] which showed that ritonavir-containing regimens were associated with higher total cholesterol (TC) and triglycerides (TG) levels and total cholesterol: high density lipoprotein cholesterol (TC: HDL-c) ratios than were indinavir-containing regimens; however, receipt of nelfinavir was associated with reduced risk of lower HDL-c levels, and receipt of saquinavir was associated with lower TC:HDL-c ratios.[7] Importantly, there is a small independent increased risk of MI in people on ART with high TG levels after adjusting for known confounders and TC and HDL cholesterol.[8] Patients receiving NNRTIs had higher levels of TC and LDL-c than did antiretroviral-naive patients, although the risk of having lower HDL-c levels was lower than that in patients receiving a single PI. Efavirenz was associated with higher levels of TC and TG than was nevirapine.[7] The 2NN study, a 48 week randomized controlled trial in ART-naïve patients randomized to efavirenz or nevirapine, also demonstrated that NNRTI containing regimens, especially nevirapine, were

associated with larger increases in HDL cholesterol and lower TC: HDL ratios. Therefore, PI-sparing regimens based on NNRTIs, especially nevirapine, are expected to result in lower coronary heart disease risk.[9]

It is important to note that describing causality of dyslipidaemia in a context of HIV is difficult. HIV infection itself can cause alterations in serum lipids.[10,11] Typically, untreated patients with advanced disease evidenced by high viral load and longer duration of infection, will have low total-, LDL-, HDL-cholesterol and high triglyceride concentrations. This is thought to be a direct effect of the HIV infection.[12] When starting ART, examining the baseline lipids and choosing the least atherogenic regimen in important.

Antiretroviral drugs and insulin resistance or dysglycaemia

Combination ART has also been associated with the increased incidence of new onset diabetes in HIV infected adults.[13] The DAD study identified stavudine and zidovudine as the drugs associated with the highest incidence of new onset diabetes, and this was attributed to their ability to cause mitochondrial toxicity leading to insulin resistance.[13] Insulin resistance in treated HIV is also associated with use of indinavir, full dose ritonavir (but not boosting doses), lopinavir, but not atazanavir.[14] Studies in healthy volunteers report conflicting results regarding the association between lopinavir and insulin resistance.[15,16] Even though efavirenz and nevirapine are not associated with decreased insulin sensitivity, marginal increases in fasting glucose have been associated with efavirenz use.[14,17] The incidence of insulin resistance with or without hyperglycaemia is reportedly higher in treated HIV infected patients compared to the general population.[18] Although the prevalence of insulin resistance in HIV is reported to range between 35% and 47% with PI therapy, with the prevalence of hyperglycaemia ranging between 3-5% and diabetes occurring in only 1%,[18] this cannot be regarded as a class effect.[17]

Hyperglycaemia or insulin resistance forms part of the metabolic syndrome. The metabolic syndrome is a term used to describe the clustering of risk factors for cardiovascular disease,

including elevated TG, low HDL, hypertension, hyperglycaemia/ insulin resistance and intra-abdominal obesity.[19] In the DAD study group, at baseline, 2439/33347 (7.3%) individuals met the criteria for the metabolic syndrome.[20] However, the prevalence has been reported to be as high as 45%, depending on the criteria used for the definition.[19] The association between metabolic syndrome and antiretroviral therapy is unclear, as individual drugs contribute to its components. For example, first generation protease inhibitors are known to cause high TG, whereas efavirenz and nevirapine increase LDL as well as HDL.[19] However, there is an increase in prevalence of the metabolic syndrome with prolonged therapy. Of importance, other risk factors such as age, sex, smoking should be taken into account when risk assessment is performed in HIV infected patients.[19]

Antiretroviral drugs and altered fat body distribution

Altered fat distribution (loss of subcutaneous fat and a relative increase in central fat) is another important complication seen in patients on long term ART.[21] The prevalence rates vary widely, 11-83%, in cross-sectional studies, and increasing rates are thought to be related to NRTI and PI use.[21] A systematic review concluded that there is a causal relationship between NRTIs (especially thymidine analogues) and lipoatrophy, with efavirenz causing an additive effect.[22] By contrast, central fat gain appears to be a consequence of treating HIV infection, because it does not differ between those treated with ART and controls, is not linked to any antiretroviral class, and doesn't improve on switching.[22] An observational study reported that altered fat distribution (lipoatrophy alone, fat gain alone, or mixed lipodystrophy phenotypes) is significantly associated with an increase cardiovascular risk.[23]

Antiretroviral drugs and hyperlactataemia

One of the important ART related complications that has impacted the way we prescribe ART is a rise in serum lactate, with or without acidosis.[24] Hyperlactataemia, associated with use of nucleoside analogue reverse transcriptase inhibitors (NRTIs), is not a single entity but a spectrum

of abnormalities.[25] The presentation varies from the common asymptomatic or symptomatic hyperlactataemia to a rare lactic acidosis, which has a high mortality.[25] In addition to other complications attributed to NRTI use, such as lipoatrophy, peripheral neuropathy and other metabolic abnormalities, the World Health Organization has since advocated for the discontinuation of use of stavudine and didanosine.[4] Where stavudine continues to be used, a reduction of dose has been advocated as improvement in metabolic parameters, such as lipids has been demonstrated.[26] However, a recently published Cochrane systematic review has not identified a clear efficacy or safety advantage of stavudine dose reduction.[27]

Antiretroviral drugs and peripheral neuropathy

Distal sensory polyneuropathy (DSP), including both antiretroviral toxic neuropathy and primary HIV sensory neuropathy, is one of the most common neurologic complications of HIV infection.[28] DSP causes pain and diminished exercise tolerance, resulting in reduced quality of life, disability and often requiring chronic analgesic use. Studies performed during the era prior to ART showed that the risk and severity of DSP was associated with advancing immunosuppression (lower CD4 cell count) and increased plasma HIV viral load.[29] However, despite improvements in immune function and viral suppression with ART, DSP remains common.[28] Although certain components of ART, specifically dideoxynucleoside drugs (stavudine and didanosine: “d-drugs”) may account for persisting DSP in some, the use of these drugs has declined, suggesting that stavudine or didanosine associated neuropathy cannot fully explain the increasing numbers of individuals experiencing DSP. The pathogenesis of “d-drug” associated neuropathy is thought to be the inhibition of DNA polymerase gamma, with subsequent mitochondrial DNA depletion.[30] PIs have not been shown to be associated with an increased risk of DSP.[28] Other risk factors, such as substance abuse, genetic predisposition, low iron, and low haemoglobin have been identified as potential contributors to DSP.[31-34]

These data reviewed above are from high-income countries and may not be generalizable to the Sub-Saharan population, which carries the highest burden of disease. For example, the DAD study cohort consisted of 24% female, median age 39, median CD4 count 430, 6% black, 24% heterosexual HIV acquisition. The protease inhibitors used at the time were nelfinavir, saquinavir, indinavir, which are no longer recommended due to poor efficacy or high toxicity. At the time ART was made available to South Africa, saquinavir, nelfinavir and indinavir were no longer the protease inhibitors of choice for the management of HIV infection and the demographics of most study populations differ. In Sub-Saharan region, the majority of patients included in the studies are younger black women with relatively lower CD4 counts at baseline, whereas, the cohorts from the Europe, Australia and United States consists mainly of white men. With this in mind, it is essential to highlight the data from the Sub-Saharan region, where the majority of the people living with HIV reside.

Studies conducted in Sub-Saharan region

The International Database to Evaluate AIDS (IeDEA) described temporal trends in baseline characteristics, initial regimens and monitoring of patients starting ART in resource-limited settings.[35] They analyzed data from 17 cohorts including 12 countries in sub-Saharan Africa, South America and Asia, between 1996-2006. In line with the WHO guidelines at the time, the majority of patients living in sub-Saharan Africa were started on ART consisting of stavudine, lamivudine and nevirapine and the median baseline CD4 count was below 200cells/μL (lowest in 2002 median 60 cells/μL and it increased to median of 122 cells/μL). In this region, nearly two thirds were younger females with a median age of 35 years at ART initiation.[35] Initially, one of the challenges faced by the antiretroviral access programs in sub-Saharan Africa was high early mortality (up to 26% in the first year of ART, with most death occurring in the first few months) observed in adults accessing treatment.[36] During this era, patients typically accessed treatment with advanced symptomatic disease, and mortality was strongly associated with baseline CD4

count less than 50 cells/μL and WHO stage 4 disease (AIDS).[36] With early diagnosis and improved access to earlier treatment, the morbidity and mortality in sub-Saharan Africa has also improved, with the AIDS related deaths reported to have declined by 48% between 2004 and 2014.[2]

With the improved longevity with ART, cumulative exposure has been associated with toxicity. The African Partnership for Chronic Disease Research study group, which published a systematic review and meta-analysis from 49 published and 3 unpublished studies, included data from 29755 adult Black individuals (5586 with individual level data) who were either HIV infected (on ART or untreated) or HIV uninfected from 14 trial sites sub-Saharan region.[37] They reported that HIV infection was associated with higher TG concentrations in univariate analyses, or after adjusting for data clustering, ART exposure, age, sex, body mass index (BMI), education level, and smoking status.[37] HIV infection was associated with lower systolic and diastolic blood pressure. Interestingly, in the HIV infected individuals, ART use was associated with lower TG concentrations and lower HbA1c. In multivariate analyses adjusting for data clustering, age, sex, body mass index (BMI), education level, and smoking status, ART was associated with higher LDL and HDL cholesterol.[37] The authors couldn't delineate the risk to individual ART drugs or drug class, but speculate that some of these findings differ from DAD study findings, which showed that ART is associated with higher TG concentrations, because there were fewer individuals on protease inhibitors in this meta-analysis.[37]

Access to antiretroviral drugs in certain parts of South Africa was made possible by non-governmental organizations prior to the rapid access antiretroviral rollout by the National Department of Health of South Africa in 2004. In line with the WHO recommendations at the time, only two treatment regimens were recommended: an initial NNRTI based regimen, followed by a PI-based regimen. Until April 2010, the first-line regimen in South Africa consisted of stavudine, lamivudine and nevirapine or efavirenz. The second-line regimen consisted of

zidovudine, didanosine and ritonavir-boosted lopinavir. Even though the majority of patients tolerated their first-line regimen well, twenty eight percent of patients had substitution in the first 3 years.[38] The cumulative incidence of stavudine associated grade 3 or 4 toxicities (peripheral neuropathy, symptomatic hyperlactataemia, and lipodystrophy) increased over time, with 21% of individuals stopping stavudine within 3 years because of toxicity.[38] Other reasons for substitutions in this study were related to zidovudine related haematological toxicity, NNRTI hypersensitivity and contraindications related to newly diagnosed tuberculosis and potential drug-drug interactions.[38] As stavudine is being currently phased out as recommended, by WHO, the currently recommended first line regimen consists of tenofovir, emtricitabine and efavirenz,[4] with the second line treatment that consist of zidovudine, lamivudine and ritonavir-boosted lopinavir.

Efavirenz is extensively prescribed and is included in the World Health Organization's preferred first-line ART regimens for HIV-1 infected adults, adolescents and children at least 3 years of age.[4] Efavirenz-based ART has been associated with the development of dyslipidaemia;[7,39,40] specifically increases in total cholesterol: HDL cholesterol ratio, LDL cholesterol, and triglycerides.[9,41] The prevalence rates of dysglycaemia were found to be similarly high in the ART naïve and treated South African patients.[42] High prevalence of both dyslipidaemia and dysglycaemia has been reported from other African countries.[43-45] However, within the treated HIV patients, the prevalence of dysglycaemia almost tripled in the group that was treated with the efavirenz-based first line regimen compared with nevirapine-based first line regimen.[42]

NRTI-related mitochondrial toxicity attributed to the inhibition of mitochondrial DNA polymerase gamma and subsequent mitochondrial dysfunction by "d-drugs", has been described as the molecular mechanism of many of these metabolic effects (lipoatrophy, hyperlactactaemia, peripheral neuropathy, dyslipidaemia and insulin resistance).[46-48] In addition, the acute inhibition of the glucose transporter GLUT4 by first generation PIs at therapeutic concentrations is also well characterized.[49] However, understanding these complications is challenging. For example, it is

known that the development of adverse events is not a class effect, but these adverse events are associated with the individual drugs.[50] Several studies have investigated the pharmacokinetic-pharmacodynamics relationships of ART associated adverse events, or genetic predisposition and the development of the ART associated adverse events. These are discussed below.

PHARMACOKINETIC-PHARMACODYNAMIC ASSOCIATIONS OF ART-ASSOCIATED METABOLIC ADVERSE EVENTS

Even though the association between antiretroviral drugs and metabolic adverse events is well described, there is paucity of data linking the adverse events to antiretroviral drug concentrations. Therapeutic drug monitoring (TDM) was proposed to optimize response to ART, improve virologic suppression, and minimize toxicity. However, some antiretroviral drugs, such as NRTIs, are unsuitable for TDM due to the poor correlation between the plasma concentrations and the active intracellular phosphorylated anabolites. A systematic review that identified 8 randomized controlled trials found no evidence to support routine TDM in all patients on either boosted PI or NNRTIs.[51] The authors concluded that TDM might be of value in patients on unboosted PIs.[51] This section will focus on the literature review on the association between efavirenz and lopinavir and metabolic complications.

Efavirenz concentrations and metabolic complications

Efavirenz-based ART has been associated with the development of dysglycaemia[42] and dyslipidaemia;[7,39,40] specifically increases in total cholesterol: HDL cholesterol ratio, LDL cholesterol, and triglycerides.[9,41] The pathogenesis of these metabolic effects are unclear, although it has been suggested that efavirenz may contribute to mitochondrial toxicity caused by concomitant thymidine analogue nucleoside reverse transcriptase inhibitors (NRTI).[47] There is contradictory, albeit limited, data regarding the association of efavirenz plasma concentrations with lipids and glucose **(Table 1).**[52-55]

There is considerable interindividual variability in plasma efavirenz exposure, which is largely explained by three *CYP2B6* loss-of-function polymorphisms.[56,57] The two polymorphisms with the greatest effect, *CYP2B6* 516G→T and *CYP2B6* 983T→C, are particularly frequent with African ancestry.[58,59] This, in part, explains why efavirenz levels concentrations are higher in patients of African ancestry.[56]

In vitro studies have demonstrated that efavirenz toxicity may be attributed to concentration dependent mitochondrial toxicity, a mechanism independent of polymerase gamma inhibition.[60-62] With the reported associations between efavirenz and dyslipidaemia and dysglycaemia, we hypothesized that, in a populations where a high proportion of patients are likely to have high concentrations, higher plasma efavirenz concentrations would be associated with higher lipid and glucose concentrations. We investigated whether plasma efavirenz concentrations correlated with plasma lipid and/or glucose concentrations in HIV-infected South Africans. We also explored if these associations are associated with *CYP2B6* genetic polymorphisms.

Table 1. Studies investigating efavirenz concentrations and metabolic parameters

Study	Participants	Study design	Objectives	Outcomes	Comments
Autar et al,[52]	59 failing NRTI based therapy and switching to ritonavir boosted indinavir and efavirenz.	Prospective 96 week longitudinal study.	To investigate associations between drug concentrations (efavirenz concentrations, indinavir and ritonavir concentration ratios (CR)) and metabolic disturbances (triglycerides, TC, LDL and HDL cholesterol) and body fat distribution.	No significant correlations efavirenz and HDL cholesterol or triglycerides. Ritonavir CR was negatively associated with HDL cholesterol. No correlations between indinavir CR and outcomes.	No adjusting for other drug concentrations was done, therefore cannot determine if independent associations were found. Only 34 participants had data at 96 weeks.
Pereira et al,[53]	34 ART naïve or PI-experienced participants starting efavirenz.	36 months Prospective longitudinal study.	To investigate effects of efavirenz on TC, HDL, LDL and LDL and their correlation to efavirenz plasma concentrations.	Positive correlation between plasma efavirenz concentrations and HDL cholesterol, or negative correlation with TC/HDL ratio after 12 months.	Small sample size, but number of samples taken over 100. Relation between plasma efavirenz concentrations and LDL cholesterol and triglycerides not reported.

Clevenbergh et al,[54]	252 patients	32 week prospective study.	To investigate impact of various PIs and NNRTI plasma concentrations on lipid abnormalities.	Use of efavirenz was associated with higher (grade 3-4 toxicity) total cholesterol (grade 3-4 toxicity). However, no correlation between NNRTI trough concentrations and lipids was found.	
Parienti et al,[55]	37 patients with mean duration of EFV exposure of 40 months and LDL-dyslipidaemia were randomly switched to nevirapine (n=18), or remain on EFV (n=19).	52 week open label randomized study.	To investigate whether switching patients from EFV to NVP would decrease LDL cholesterol levels and whether there is dose-effect relationship.	Switching from EFV to NVP was associated with a significant decrease in LDL cholesterol. NVP plasma concentrations were associated with the decrease in LDL cholesterol concentrations. There was no significant change in the EFV arm. No association between EFV concentrations and LDL cholesterol	Small study that only recruited patients with LDL dyslipidaemia.

Lopinavir concentrations and metabolic complications

Ritonavir boosted lopinavir (LPV/r) is a potent protease inhibitor (PI) widely used in a second-line antiretroviral regimens in low and middle-income countries.[4] Treatment with LPV/r has been associated with the development of dyslipidaemia, which is a known risk factor for cardiovascular complications.[13,63] A systematic review including 15 trials and 6367 participants showed that ritonavir-boosted lopinavir had a similar effect on total cholesterol when compared with efavirenz.[64] However, triglycerides were higher in the ritonavir-boosted PI arms compared with efavirenz.[64]

There are conflicting results regarding an association between LPV/r use and the development of insulin resistance and new onset diabetes. [16,47,65-67] Furthermore, there is contradictory data as to whether the metabolic toxicity is associated with higher plasma concentrations of either lopinavir or ritonavir.[54,68-71] **Table 2** illustrates the different studies that have investigated the association between the lopinavir or ritonavir concentrations and metabolic parameters.

Lopinavir is metabolized primarily by hepatic cytochrome P450 (CYP) 3A isoforms and is a substrate for p-glycoprotein (P-gp).[72] Its co-formulation with ritonavir increases lopinavir exposure by inhibiting intestinal and hepatic CYP3A-mediated and P-gp-mediated drug metabolism and transport, respectively.[72] Recently, protease inhibitors have been reported to be substrates of the drug transporter belonging to the organic anion transporting polypeptide (OATP/SLCO) family.[72] Lopinavir has large interindividual PK variability, at least part of which can be explained by age, sex, body weight, drug-drug interactions, liver disease, pregnancy and poor-adherence.[72] The remaining variability is attributed to host genetic factors.

Limited studies have found no association between LPV plasma concentrations and polymorphisms in ABCB1, CYP3A5, CYP2B6 and CYP2D6 genes.[73] However, in HIV infected men, recent studies have reported “gain of function” (rs11045819) and “loss of function” (rs4149056) variants in *SLCO1B1* gene, which may lead to low and high plasma concentrations

of LPV, respectively.[72,73] In our population, where the prevalence of HIV and tuberculosis co-infection is high, concomitant administration of lopinavir and rifampin based antituberculous therapy is common. Furthermore, a recent study found an association between an *SLCO1B1* variant (rs4149032), a highly prevalent polymorphism, and low rifampicin exposure.[74]

Widespread long-term PI use may be limited by these metabolic complications. We investigated whether plasma lopinavir concentrations correlated with plasma lipid and/or glucose concentrations in HIV-infected South Africans. As lopinavir has a high interindividual variability, which may be explained by genetic polymorphisms,[72,73,75,76] we hypothesized higher plasma lopinavir concentrations would be associated with higher lipid and glucose concentrations.

Table 2. Studies investigating lopinavir concentrations and metabolic parameters

Study	Participants	Study design	Objectives	Outcomes	Comments
Clevenbergh[54]	252 patients	32 week prospective study	To investigate impact of various PIs and NNRTI plasma concentrations on lipid abnormalities	Use of lopinavir ritonavir was associated with higher (grade 3-4 toxicity) total cholesterol and triglycerides. However, no correlation between lopinavir or ritonavir trough concentrations and lipids was found.	
de Requena et al,[68]	126 participants starting salvage therapy with lopinavir ritonavir	3 months prospective longitudinal study	To investigate association between lopinavir or ritonavir plasma concentrations and fasting triglycerides or total cholesterol	Significant increases in both triglycerides and total cholesterol from baseline to month 3. A positive correlation between LPV C_{trough} and percentage increase in triglyceride, but not cholesterol. A positive correlation between ritonavir C_{trough} and percentage increase in cholesterol was observed.	Heavily pretreated
Guitérrez et al,[69]	22 patients with	48 week Prospective	To investigate association between LPV plasma	Significant associations between higher LPV trough concentrations and	Only 19 and 16 completed 24

	virologic failure on PI therapy	longitudinal study	concentrations and lipids	hypertriglyceridaemia and hypercholesterolaemia.	and 48 weeks.
Leon et al,[70]	26 ART experienced participants starting lopinavir	24 week longitudinal study	To investigate correlation between fasting plasma lipids and body fat (DEXA scan) and plasma lipids and lopinavir plasma concentrations	There was an increase in plasma triglycerides and total cholesterol and limb fat gain at 24 weeks, but there was no change in glucose concentrations. There was no correlation with lopinavir plasma concentrations (AUC_{12} C_{max}, C_{trough}) and metabolic parameters (triglycerides, glucose or total, HDL cholesterol or LDL cholesterol serum levels) or body fat.	Small study. Only 20 subjects had 24-week data.
Torti et al, [71]	55 patients on ritonavir-boosted lopinavir	12 week longitudinal study	To investigate whether lipid changes are associated with lopinavir plasma concentration	In multivariate analyses LPV AUC was not associated with changes in the AUC of cholesterol or triglycerides at 4 or 12 weeks	Not clear what covariates were adjusted for.

Rhee[77]	25 patient, PI naïve starting saquinavir/lopinavir ritonavir	Retrospective 12 week longitudinal study at 1, 4 and 12 weeks	To investigate whether lipid changes are associated with LPV plasma concentrations	Significant increases in both triglycerides and total cholesterol from baseline to week 12. No associations between lopinavir and lipids	Small study
Bierman[78]	82 patients ART naïve starting lopinavir therapy	Prospective study lopinavir based therapy at different doses	To determine the relationship between drug concentration and lipid changes in two patient cohorts.	Significant increases in both triglycerides and total cholesterol from baseline to week 552 days. No associations between lopinavir and lipids. No difference between the comparison arms	Nevirapine and lopinavir/ritonavir (533/133 mg) vs. zidovudine, lamivudine and lopinavir/ritonavir (400/100 mg)
Ter Hofstede[79]	72 subjects ART naïve on lopinavir for 12-24	24 week multicenter prospective cohort	To determine the relationship between lopinavir concentrations and lipid changes	No associations between lopinavir plasma concentrations and triglycerides or cholesterol (change from baseline to 24 weeks)	Wide intra-individual variability

PHARMACOGENOMICS OF ANTIRETROVIRAL DRUGS

Genetic polymorphisms have been associated with interindividual variability of antiretroviral drug disposition, ART related toxicities and ageing related complications of HIV. Therefore, pharmacogenomics may identify patients at increased risk for toxicity and/or reduced efficacy of antiretroviral therapy. This review will focus on the pharmacogenomics efavirenz and mitochondrial genomics of NRTI associated metabolic complications.

Efavirenz pharmacogenomics

Efavirenz is metabolized primarily by cytochrome P450 (CYP) 2B6.[80] The metabolic pathway of efavirenz is displayed in Figure 1.[81] Efavirenz is extensively prescribed for HIV-1 infection worldwide. While generally safe and effective, some efavirenz recipients experience virologic failure,[82] and/or central nervous system symptoms.[83] There is considerable interindividual variability in plasma efavirenz exposure, which is largely explained by CYP2B6 loss-of-function polymorphisms.[56,57] Analyses with AIDS Clinical Trials Group (ACTG) protocol 5097s first showed that the non-synonymous polymorphism *CYP2B6* 516G→T (rs3745274) was strongly associated with increased plasma efavirenz exposure.[56] Many studies have since replicated this association.[84-87] The *CYP2B6* 516 T allele is more frequent with African ancestry than with European ancestry,[58] which largely explains the greater mean plasma efavirenz concentrations reported among populations of African descent.[88,89] The *CYP2B6* 516G→T polymorphism is also frequent in Thai and Cambodian populations.[90,91]A considerably less frequent *CYP2B6* polymorphism, 983T→C (rs28399499), also predicts increased plasma efavirenz exposure.[92-94] The *CYP2B6* 983 C allele is found almost exclusively with African ancestry, among whom it is still much less frequent than 516G→T.[95] Therefore, the two polymorphisms with the greatest effect, *CYP2B6* 516G→T and *CYP2B6* 983T→C, are particularly frequent in people of African ancestry.[58,59]

Additional *CYP2B6* polymorphisms suggested to affect *CYP2B6* activity have been extremely infrequent,[92,96,97] or have not predicted plasma efavirenz exposure.[97,98] Polymorphisms in genes beyond *CYP2B6* reported to affect interindividual variability in efavirenz pharmacokinetics include *CYP2A6*,[99] *CYP3A5*,[56] *UGT2B7*,[100] and *CAR*,[101] although such associations have yet to be replicated. However, in a recent genome-wide associations study involving White, Black, and Hispanic adults in the United States, only *CYP2B6* 516G→T, 983T→C, and an intronic *CYP2B6* variant (rs4803419) were independently associated with estimated efavirenz trough concentrations (P=4.4x10-15).[57]

Previous studies from Africa have replicated the association between CYP2B6 516G→T and plasma efavirenz exposure, including studies of patients from Zimbabwe [102-104], South Africa [105-108], Ghana [109,110], Uganda [104,111], Tanzania [112,113], Rwanda [114], and Ethiopia [112]. Multiple studies of patients from Africa have also shown the association with 983T→C [59,103,104,108,110,114]. Data for association beyond CYP2B6 are limited. A study of patients in Ghana found associations with CYP2A6 and UGT2B7 polymorphisms [109], but a subsequent study of patients in Ghana did not replicate independent associations with these polymorphisms [110].

We characterized relationships between candidate genetic polymorphisms and plasma efavirenz concentrations among HIV-infected adults and children in South Africans.

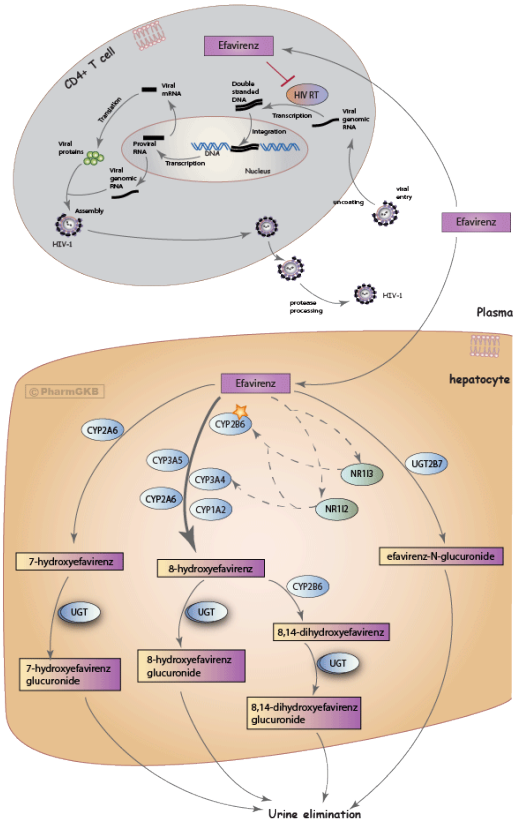

Figure 1. Efavirenz metabolic pathway. The metabolic pathway of efavirenz showing various genes involved in the efavirenz disposition. The star represents significant. The blue oval circles represent generic genes, greyish oval circle represents downstream target gene, purple rectangle represent the drug. Mixed purple and yellow rectangle represents metabolite. The main metabolite is 8-hydroxyefavirenz. *CYP2B6*= gene coding for cytochrome P450 isoenzyme 2B6. UGT= UDP-glucuronosyltransferase, transcription factors pregnane X receptor (PXR, *NR1I2*) and constitutive androstane receptor (CAR, *NR1I3*), *NR1I2/NR1I3*= gene coding for nuclear receptor subfamily 1, group I member 2/3 (reproduced by permission from the PharmGKB and Stanford University (url: https://www.pharmgkb.org/pathway/PA166123135)

Mitochondrial haplogroups and metabolic complications and peripheral neuropathy

Exposure to ART has been associated with metabolic adverse effects such as dyslipidaemia, insulin resistance, dysglycaemia, central fat accumulation, peripheral fat loss (lipoatrophy), and peripheral neuropathy.[30,48] Many of these adverse effects are thought to be related to NRTI-related mitochondrial toxicity, at least in part due to inhibition of mitochondrial DNA (mtDNA) gamma polymerase and subsequent mitochondrial dysfunction.[30] Other antiretroviral classes have also been associated with mitochondrial dysfunction.[46,115]

Mitochondrial DNA is distinct from nuclear DNA and codes for 13 polypeptides essential for oxidative phosphorylation.[116] Mitochondrial DNA exhibits abundant genetic variation across the 16.6 kb mitochondrial genome. Human mtDNA sequences have diverged over approximately the last 150,000 years due to natural selection and human migration, resulting in distinct patterns of single nucleotide polymorphisms (SNPs), called haplogroups.[117] **Figure 2** shows the simplified phylogenetic tree (www.phylotree.org).[118]

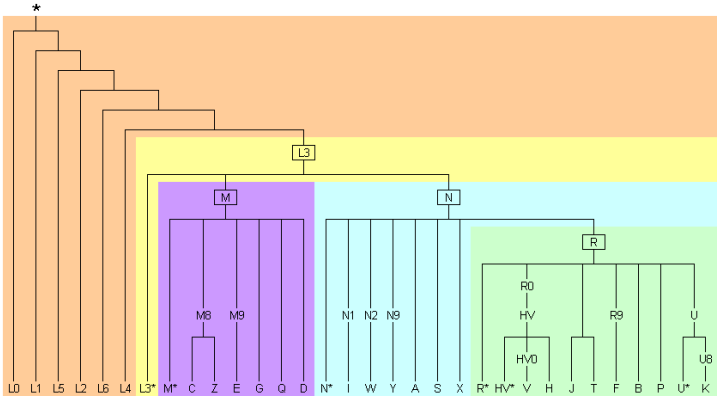

Figure 2. Simplified mtDNA phylogeny illustrating the use of alphabetical letters for haplogroup designation. A star representing the most recent common matrilineal ancestor of all humans indicates the root of the tree. The L haplogroups are the most deep-rooting lineages and are African specific indicating the African origin of modern humans. Haplogroup L3 gave rise to macrohaplogroups M, N and R, which encompass all variation observed outside Africa.

Studies in HIV-uninfected populations (primarily European and Asian) have reported associations between mtDNA and metabolic complications including dyslipidaemia and diabetes.[119-123] A recent systematic review summarized studies of significant associations between mitochondrial haplogroups with HIV and /or ART-related complications potentially having mitochondrial mechanisms.[124] Evidence for significant associations between European mtDNA haplogroups and outcomes in HIV infected participants has been reported for CD4 count recovery,[125] AIDS progression,[126] and ART related complications (including lipoatrophy,[127-130] insulin resistance,[131,132] dyslipidaemia,[128] atherogenic risk,[131] peripheral neuropathy,[31,133,134] hepatic fibrosis/cirrhosis,[135] and neuroretinal disorders[136]).

The majority of HIV-infected persons worldwide reside in sub-Saharan Africa,[1] but this region has been under-represented in genetic studies. Studies in African Americans showed conflicting results regarding associations between African mtDNA sub-haplogroup L1c and peripheral neuropathy,[137,138] and a positive association between haplogroup L2 and $CD4^+$ T-cell recovery.[125,139] A study conducted in Malawi found that the African mtDNA subhaplogroup L02a increased the susceptibility to peripheral neuropathy, whereas subhaplogroup L2a was protective. At the time of conducting our study, there were no published studies that investigated associations between African mtDNA haplogroups and important metabolic complications including lipoatrophy, dysglycaemia or dyslipidaemia. Subsequently, a small study (n=37) conducted in South Africans showed no association between the African mtDNA haplogroups and hyperlactataemia.[140] An updated table of studies including HIV-infected patients of African ancestry is summarized in Table 3. The discrepancy with different associations might be attributed to different mtDNA haplogroup frequencies found in different study populations. For example, in African Americans the frequent haplogroups are L1, L2, L3 (increasing order),[141] and in the Malawians, the frequent haplogroups were L1, L3, L2 and L0 (increasing order).[142]

In this study, we report the prevalence of mtDNA haplogroups in a South African population enrolled in an ART program, and explore associations between African mtDNA and ART-associated complications including metabolic complications and distal sensory polyneuropathy (DSP). Because ART-associated adverse effects in HIV-infected persons, and metabolic diseases in general, are believed to result in part from mitochondrial dysfunction, we hypothesized that mtDNA variation would be associated with susceptibility to ART-associated lipoatrophy, dysglycaemia, hypertriglyceridaemia and/or distal peripheral neuropathy in this South Africa.

Table 3. Studies investigating associations between African mitochondrial DNA haplogroups and ART related complications.

		African mitochondrial DNA haplogroup			
Study	Phenotype	L0	L1	L2	L3
Canter[137*]	Peripheral neuropathy		(L1c) ↑		
Holzinger[138*]	Peripheral neuropathy		(L1c)↓		
Kampira[142#]	Peripheral neuropathy	(L0a2)↑		(L2a)↓	
Aissani[139*]	$CD4^+$ cell recovery			↓	
Grady[125*]	$CD4^+$ cell recovery			↓	
Hulgan[143*]	$CD4^+$ cell recovery			↓	
Hulgan[141*]	Neurocognitive impairment		⇔	⇔	⇔
Arenas-Pinto[140$]	Hyperlactataemia	⇔		⇔	⇔

[*]Studies conducted in African-Americans, [#] study conducted in Malawians, [$]study conducted in South Africans. Unless specified, the arrow showing the direction of the association refers to the major haplogroup. ↑ means increased risk, ↓ means protective effect, ⇔ means no associations found with any of the African mtDNA haplogroups and the phenotype of interest.

Bibliography and References Cited

1. UNAIDS. 90-90-90 An ambitious treatment target to help end AIDS epidemic. 2014; http://www.unaids.org/en/resources/documents/2014/90-90-90.
2. UNAIDS. How AIDS changed everything. MGD 6: 15 years, 15 lessons of hope from the AIDS response. 2015; http://www.unaids.org/en/resources/documents/2015/20150714_factsheet.
3. Shisana O RT, Simbayi LC, Zuma K, Jooste S, Zungu N, et al. *South African National HIV Prevalence, Incidence and Behaviour Survey,2012*. Cape Town: HSRC Press;2014.
4. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and prenting HIV infection. 2013; http://www.who.int/hiv/pub/guidelines/arv2013/download/en/.
5. Friis-Moller N, Sabin CA, Weber R, et al. Combination antiretroviral therapy and the risk of myocardial infarction. *N Engl J Med.* 2003;349(21):1993-2003.
6. Friis-Moller N, Weber R, Reiss P, et al. Cardiovascular disease risk factors in HIV patients--association with antiretroviral therapy. Results from the DAD study. *AIDS.* 2003;17(8):1179-1193.
7. Fontas E, van Leth F, Sabin CA, et al. Lipid profiles in HIV-infected patients receiving combination antiretroviral therapy: are different antiretroviral drugs associated with different lipid profiles? *J Infect Dis.* 2004;189(6):1056-1074.
8. Worm SW, Kamara DA, Reiss P, et al. Elevated triglycerides and risk of myocardial infarction in HIV-positive persons. *AIDS.* 2011;25(12):1497-1504.
9. van Leth F, Phanuphak P, Stroes E, et al. Nevirapine and efavirenz elicit different changes in lipid profiles in antiretroviral-therapy-naive patients infected with HIV-1. *PLoS Med.* 2004;1(1):e19.
10. Anastos K, Lu D, Shi Q, et al. Association of serum lipid levels with HIV serostatus, specific antiretroviral agents, and treatment regimens. *J Acquir Immune Defic Syndr.* 2007;45(1):34-42.

11. Riddler SA, Smit E, Cole SR, et al. Impact of HIV infection and HAART on serum lipids in men. *Jama.* 2003;289(22):2978-2982.

12. El-Sadr WM, Mullin CM, Carr A, et al. Effects of HIV disease on lipid, glucose and insulin levels: results from a large antiretroviral-naive cohort. *HIV Med.* 2005;6(2):114-121.

13. De Wit S, Sabin CA, Weber R, et al. Incidence and risk factors for new-onset diabetes in HIV-infected patients: the Data Collection on Adverse Events of Anti-HIV Drugs (D:A:D) study. *Diabetes Care.* 2008;31(6):1224-1229.

14. Feeney ER, Mallon PW. Insulin resistance in treated HIV infection. *Best Pract Res Clin Endocrinol Metab.* 2011;25(3):443-458.

15. Noor MA, Flint OP, Maa JF, Parker RA. Effects of atazanavir/ritonavir and lopinavir/ritonavir on glucose uptake and insulin sensitivity: demonstrable differences in vitro and clinically. *AIDS.* 2006;20(14):1813-1821.

16. Dube MP, Shen C, Greenwald M, Mather KJ. No impairment of endothelial function or insulin sensitivity with 4 weeks of the HIV protease inhibitors atazanavir or lopinavir-ritonavir in healthy subjects without HIV infection: a placebo-controlled trial. *Clin Infect Dis.* 2008;47(4):567-574.

17. Erlandson KM, Kitch D, Tierney C, et al. Impact of randomized antiretroviral therapy initiation on glucose metabolism. *AIDS.* 2014;28(10):1451-1461.

18. Aboud M, Elgalib A, Kulasegaram R, Peters B. Insulin resistance and HIV infection: a review. *International journal of clinical practice.* 2007;61(3):463-472.

19. Worm SW, Lundgren JD. The metabolic syndrome in HIV. *Best Pract Res Clin Endocrinol Metab.* 2011;25(3):479-486.

20. Worm SW, Friis-Moller N, Bruyand M, et al. High prevalence of the metabolic syndrome in HIV-infected patients: impact of different definitions of the metabolic syndrome. *AIDS.* 2010;24(3):427-435.

21. Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N Engl J Med.* 2005;352(1):48-62.

22. de Waal R, Cohen K, Maartens G. Systematic review of antiretroviral-associated lipodystrophy: lipoatrophy, but not central fat gain, is an antiretroviral adverse drug reaction. *PLoS One.* 2013;8(5):e63623.

23. Guaraldi G, Stentarelli C, Zona S, et al. Lipodystrophy and anti-retroviral therapy as predictors of sub-clinical atherosclerosis in human immunodeficiency virus infected subjects. *Atherosclerosis.* 2010;208(1):222-227.

24. John M, Mallal S. Hyperlactatemia syndromes in people with HIV infection. *Curr Opin Infect Dis.* 2002;15(1):23-29.

25. Imhof A, Ledergerber B, Gunthard HF, Haupts S, Weber R. Risk factors for and outcome of hyperlactatemia in HIV-infected persons: is there a need for routine lactate monitoring? *Clin Infect Dis.* 2005;41(5):721-728.

26. Milinkovic A, Martinez E, Lopez S, et al. The impact of reducing stavudine dose versus switching to tenofovir on plasma lipids, body composition and mitochondrial function in HIV-infected patients. *Antivir Ther.* 2007;12(3):407-415.

27. Magula N, Dedicoat M. Low dose versus high dose stavudine for treating people with HIV infection. *The Cochrane database of systematic reviews.* 2015;1:Cd007497.

28. Ellis RJ, Marquie-Beck J, Delaney P, et al. Human immunodeficiency virus protease inhibitors and risk for peripheral neuropathy. *Annals of neurology.* 2008;64(5):566-572.

29. Schifitto G, McDermott MP, McArthur JC, et al. Incidence of and risk factors for HIV-associated distal sensory polyneuropathy. *Neurology.* 2002;58(12):1764-1768.

30. Kohler JJ, Lewis W. A brief overview of mechanisms of mitochondrial toxicity from NRTIs. *Environmental and molecular mutagenesis.* 2007;48(3-4):166-172.

31. Hulgan T, Haas DW, Haines JL, et al. Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: an adult AIDS clinical trials group study. *AIDS.* 2005;19(13):1341-1349.

32. Morgello S, Estanislao L, Simpson D, et al. HIV-associated distal sensory polyneuropathy in the era of highly active antiretroviral therapy: the Manhattan HIV Brain Bank. *Archives of neurology.* 2004;61(4):546-551.

33. Robinson-Papp J, Gonzalez-Duarte A, Simpson DM, Rivera-Mindt M, Morgello S. The roles of ethnicity and antiretrovirals in HIV-associated polyneuropathy: a pilot study. *J Acquir Immune Defic Syndr.* 2009;51(5):569-573.

34. Kallianpur AR, Hulgan T. Pharmacogenetics of nucleoside reverse-transcriptase inhibitor-associated peripheral neuropathy. *Pharmacogenomics.* 2009;10(4):623-637.

35. Keiser O, Anastos K, Schechter M, et al. Antiretroviral therapy in resource-limited settings 1996 to 2006: patient characteristics, treatment regimens and monitoring in sub-Saharan Africa, Asia and Latin America. *Tropical medicine & international health : TM & IH.* 2008;13(7):870-879.

36. Lawn SD, Harries AD, Anglaret X, Myer L, Wood R. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS.* 2008;22(15):1897-1908.

37. Dillon DG, Gurdasani D, Riha J, et al. Association of HIV and ART with cardiometabolic traits in sub-Saharan Africa: a systematic review and meta-analysis. *Int J Epidemiol.* 2013;42(6):1754-1771.

38. Boulle A, Orrel C, Kaplan R, et al. Substitutions due to antiretroviral toxicity or contraindication in the first 3 years of antiretroviral therapy in a large South African cohort. *Antivir Ther.* 2007;12(5):753-760.

39. Tashima KT, Bausserman L, Alt EN, Aznar E, Flanigan TP. Lipid changes in patients initiating efavirenz- and indinavir-based antiretroviral regimens. *HIV Clin Trials.* 2003;4(1):29-36.

40. Williams P, Wu J, Cohn S, et al. Improvement in lipid profiles over 6 years of follow-up in adults with AIDS and immune reconstitution. *HIV Med.* 2009;10(5):290-301.

41. Rhoads MP, Lanigan J, Smith CJ, Lyall EG. Effect of specific ART drugs on lipid changes and the need for lipid management in children with HIV. *J Acquir Immune Defic Syndr.* 2011;57(5):404-412.

42. Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr.* 2011;57(4):284-289.

43. Zannou DM, Denoeud L, Lacombe K, et al. Incidence of lipodystrophy and metabolic disorders in patients starting non-nucleoside reverse transcriptase inhibitors in Benin. *Antivir Ther.* 2009;14(3):371-380.

44. Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2007;46(4):451-455.

45. Omech B, Sempa J, Castelnuovo B, et al. Prevalence of HIV-Associated Metabolic Abnormalities among Patients Taking First-Line Antiretroviral Therapy in Uganda. *ISRN AIDS.* 2012;2012:960178.

46. Karamchand L, Dawood H, Chuturgoon AA. Lymphocyte mitochondrial depolarization and apoptosis in HIV-1-infected HAART patients. *J Acquir Immune Defic Syndr.* 2008;48(4):381-388.

47. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy,

hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet.* 1999;353(9170):2093-2099.

48. Falutz J. HIV infection, body composition changes and related metabolic complications: contributing factors and evolving management strategies. *Curr Opin Clin Nutr Metab Care.* 2011;14(3):255-260.

49. Hresko RC, Kraft TE, Tzekov A, Wildman SA, Hruz PW. Isoform-selective inhibition of facilitative glucose transporters: elucidation of the molecular mechanism of HIV protease inhibitor binding. *The Journal of biological chemistry.* 2014;289(23):16100-16113.

50. Fellay J, Boubaker K, Ledergerber B, et al. Prevalence of adverse events associated with potent antiretroviral treatment: Swiss HIV Cohort Study. *Lancet.* 2001;358(9290):1322-1327.

51. Kredo T, Van der Walt JS, Siegfried N, Cohen K. Therapeutic drug monitoring of antiretrovirals for people with HIV. *The Cochrane database of systematic reviews.* 2009(3):Cd007268.

52. Autar RS, Boyd MA, Wit FW, et al. Relationships between drug exposure, changes in metabolic parameters and body fat in HIV-infected patients switched to a nucleoside sparing regimen. *Antivir Ther.* 2007;12(8):1265-1271.

53. Pereira SA, Branco T, Corte-Real RM, et al. Long-term and concentration-dependent beneficial effect of efavirenz on HDL-cholesterol in HIV-infected patients. *Br J Clin Pharmacol.* 2006;61(5):601-604.

54. Clevenbergh P, Garraffo R, Dellamonica P. Impact of various antiretroviral drugs and their plasma concentrations on plasma lipids in heavily pretreated HIV-infected patients. *HIV Clin Trials.* 2003;4(5):330-336.

55. Parienti JJ, Massari V, Rey D, Poubeau P, Verdon R. Efavirenz to nevirapine switch in HIV-1-infected patients with dyslipidemia: a randomized, controlled study. *Clin Infect Dis.* 2007;45(2):263-266.

56. Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS.* 2004;18(18):2391-2400.

57. Holzinger ER, Grady B, Ritchie MD, et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. *Pharmacogenet Genomics.* 2012;22(12):858-867.

58. dbSNP. Short Genetic Variations. http://www.ncbi.nlm.nih.gov/projects/SNP/. .

59. Wang J, Sonnerborg A, Rane A, et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenet Genomics.* 2006;16(3):191-198.

60. Blas-Garcia A, Apostolova N, Ballesteros D, et al. Inhibition of mitochondrial function by efavirenz increases lipid content in hepatic cells. *Hepatology.* 2010;52(1):115-125.

61. Blas-Garcia A, Polo M, Alegre F, et al. Lack of mitochondrial toxicity of darunavir, raltegravir and rilpivirine in neurons and hepatocytes: a comparison with efavirenz. *J Antimicrob Chemother.* 2014;69(11):2995-3000.

62. Polo M, Alegre F, Funes HA, et al. Mitochondrial (dys)function - a factor underlying the variability of efavirenz-induced hepatotoxicity? *Br J Pharmacol.* 2015;172(7):1713-1727.

63. Montes ML, Pulido F, Barros C, et al. Lipid disorders in antiretroviral-naive patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors. *J Antimicrob Chemother.* 2005;55(5):800-804.

64. Hill A, Sawyer W, Gazzard B. Effects of first-line use of nucleoside analogues, efavirenz, and ritonavir-boosted protease inhibitors on lipid levels. *HIV Clin Trials.* 2009;10(1):1-12.

65. Galindo MJ, Verdejo J, Gonzalez-Munoz M, Ferrer A, Polo R. Metabolic changes in protease inhibitor-naive patients treated for 1 year with lopinavir/ritonavir. *J Acquir Immune Defic Syndr.* 2008;48(5):628-629.

66. Pao VY, Lee GA, Taylor S, et al. The protease inhibitor combination lopinavir/ritonavir does not decrease insulin secretion in healthy, HIV-seronegative volunteers. *AIDS.* 2010;24(2):265-270.

67. Lee GA, Lo JC, Aweeka F, et al. Single-dose lopinavir-ritonavir acutely inhibits insulin-mediated glucose disposal in healthy volunteers. *Clin Infect Dis.* 2006;43(5):658-660.

68. Gonzalez de Requena D, Blanco F, Garcia-Benayas T, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Correlation between lopinavir plasma levels and lipid abnormalities in patients taking lopinavir/ritonavir. *AIDS patient care and STDs.* 2003;17(9):443-445.

69. Gutierrez F, Padilla S, Navarro A, et al. Lopinavir plasma concentrations and changes in lipid levels during salvage therapy with lopinavir/ritonavir-containing regimens. *J Acquir Immune Defic Syndr.* 2003;33(5):594-600.

70. Leon A, Martinez E, Sarasa M, et al. Impact of steady-state lopinavir plasma levels on plasma lipids and body composition after 24 weeks of lopinavir/ritonavir-containing therapy free of thymidine analogues. *J Antimicrob Chemother.* 2007;60(4):824-830.

71. Torti C, Quiros-Roldan E, Regazzi-Bonora M, et al. Lipid abnormalities in HIV-infected patients are not correlated with lopinavir plasma concentrations. *J Acquir Immune Defic Syndr.* 2004;35(3):324-326.

72. Lubomirov R, di Iulio J, Fayet A, et al. ADME pharmacogenetics: investigation of the pharmacokinetics of the antiretroviral agent lopinavir coformulated with ritonavir. *Pharmacogenet Genomics.* 2010;20(4):217-230.

73. Hartkoorn RC, Kwan WS, Shallcross V, et al. HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. *Pharmacogenet Genomics.* 2010;20(2):112-120.

74. Chigutsa E, Visser ME, Swart EC, et al. The SLCO1B1 rs4149032 polymorphism is highly prevalent in South Africans and is associated with reduced rifampin

concentrations: dosing implications. *Antimicrob Agents Chemother.* 2011;55(9):4122-4127.

75. Kohlrausch FB, de Cassia Estrela R, Barroso PF, Suarez-Kurtz G. The impact of SLCO1B1 polymorphisms on the plasma concentration of lopinavir and ritonavir in HIV-infected men. *Br J Clin Pharmacol.* 2010;69(1):95-98.

76. Lubomirov R, Colombo S, di Iulio J, et al. Association of pharmacogenetic markers with premature discontinuation of first-line anti-HIV therapy: an observational cohort study. *J Infect Dis.* 2011;203(2):246-257.

77. Rhee MS, Hellinger JA, Sheble-Hall S, Cohen CJ, Greenblatt DJ. Relationship between plasma protease inhibitor concentrations and lipid elevations in HIV patients on a double-boosted protease inhibitor regimen (saquinavir/lopinavir/ritonavir). *J Clin Pharmacol.* 2010;50(4):392-400.

78. Bierman WF, van Vonderen MG, Veldkamp AI, et al. The lopinavir/ritonavir-associated rise in lipids is not related to lopinavir or ritonavir plasma concentration. *Antivir Ther.* 2011;16(5):647-655.

79. Ter Hofstede HJ, Koopmans PP, Burger DM, et al. Lopinavir Plasma Concentrations and Serum Lipids in Therapy Naïve HIV-Patients: A Sub Analysis of the FREE Study. 2012.

80. Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. *J.Pharmacol.Exp.Ther.* 2003;306(1):287-300.

81. Whirl-Carrillo M, McDonagh EM, Hebert JM, et al. Pharmacogenomics knowledge for personalized medicine. *Clin Pharmacol Ther.* 2012;92(4):414-417.

82. Gulick RM, Ribaudo HJ, Shikuma CM, et al. Three- vs four-drug antiretroviral regimens for the initial treatment of HIV-1 infection: a randomized controlled trial. *JAMA.* 2006;296(7):769-781.

83. Clifford DB, Evans S, Yang Y, et al. Impact of efavirenz on neuropsychological performance and symptoms in HIV-infected individuals. *Ann.Intern.Med.* 2005;143(10):714-721.

84. Haas DW, Smeaton LM, Shafer RW, et al. Pharmacogenetics of Long-Term Responses to Antiretroviral Regimens Containing Efavirenz and/or Nelfinavir: An Adult AIDS Clinical Trials Group Study. *J.Infect.Dis.* 2005;192(11):1931-1942.

85. Rotger M, Colombo S, Furrer H, et al. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. *Pharmacogenetics Genomics.* 2005;15(1):1-5.

86. Almond LM, Hoggard PG, Edirisinghe D, Khoo SH, Back DJ. Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. *J.Antimicrob.Chemother.* 2005;56(4):738-744.

87. Rodriguez-Novoa S, Barreiro P, Rendon A, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Influence of 516G>T polymorphisms at the gene encoding the CYP450-2B6 isoenzyme on efavirenz plasma concentrations in HIV-infected subjects. *Clin Infect Dis.* 2005;40(9):1358-1361.

88. Barrett JS, Joshi AS, Chai M, Ludden TM, Fiske WD, Pieniaszek HJ, Jr. Population pharmacokinetic meta-analysis with efavirenz. *Int.J.Clin.Pharmacol.Ther.* 2002;40(11):507-519.

89. Pfister M, Labbe L, Hammer SM, et al. Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: Adult AIDS Clinical Trial Group Study 398. *Antimicrob.Agents Chemother.* 2003;47(1):130-137.

90. Chou M, Bertrand J, Segeral O, et al. Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients. *Antimicrob Agents Chemother.* 2010;54(10):4432-4439.

91. Sukasem C, Cressey TR, Prapaithong P, et al. Pharmacogenetic markers of CYP2B6 associated with efavirenz plasma concentrations in HIV-1 infected Thai adults. *Br J Clin Pharmacol.* 2012;74(6):1005-1012.

92. Wang J, Sonnerborg A, Rane A, et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenet.Genomics.* 2006;16(3):191-198.

93. Wyen C, Hendra H, Vogel M, et al. Impact of CYP2B6 983T>C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. *J Antimicrob.Chemother.* 2008;61(4):914-918.

94. Ribaudo HJ, Liu H, Schwab M, et al. Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. *J Infect Dis.* 2010;202(5):717-722.

95. dbSNP - Short Genetic Variations. 2011; http://www.ncbi.nlm.nih.gov/projects/SNP/. Accessed 9/12/2011, 2011.

96. Rotger M, Colombo S, Cavassini M, et al. Genetic Variability of CYP2B6 in Individuals with Extremely High Efavirenz Plasma Concentrations. *Presented at 13th Conference on Retroviruses and Opportunistic Infections, February 2006, Denver, CO.Abstract 572.* 2006.

97. Rotger M, Tegude H, Colombo S, et al. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. *Clin Pharmacol.Ther.* 2007;81(4):557-566.

98. Rotger M, Saumoy M, Zhang K, et al. Partial deletion of CYP2B6 owing to unequal crossover with CYP2B7. *Pharmacogenet.Genomics.* 2007;17(10):885-890.

99. di Iulio J, Fayet A, Arab-Alameddine M, et al. In vivo analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. *Pharmacogenet Genomics.* 2009;19(4):300-309.

100. Kwara A, Lartey M, Sagoe KW, Kenu E, Court MH. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. *AIDS.* 2009.

101. Wyen C, Hendra H, Siccardi M, et al. Cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) polymorphisms are associated with early discontinuation of efavirenz-containing regimens. *The Journal of antimicrobial chemotherapy.* 2011;66(9):2092-2098.

102. Nyakutira C, Roshammar D, Chigutsa E, et al. High prevalence of the CYP2B6 516G-->T(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. *European journal of clinical pharmacology.* 2008;64(4):357-365.

103. Maimbo M, Kiyotani K, Mushiroda T, Masimirembwa C, Nakamura Y. CYP2B6 genotype is a strong predictor of systemic exposure to efavirenz in HIV-infected Zimbabweans. *European journal of clinical pharmacology.* 2012;68(3):267-271.

104. Jamshidi Y, Moreton M, McKeown DA, et al. Tribal ethnicity and CYP2B6 genetics in Ugandan and Zimbabwean populations in the UK: implications for efavirenz dosing in HIV infection. *The Journal of antimicrobial chemotherapy.* 2010;65(12):2614-2619.

105. Cohen K, Grant A, Dandara C, et al. Effect of rifampicin-based antitubercular therapy and the cytochrome P450 2B6 516G>T polymorphism on efavirenz concentrations in adults in South Africa. *Antivir Ther.* 2009;14(5):687-695.

106. Gounden V, van Niekerk C, Snyman T, George JA. Presence of the CYP2B6 516G> T polymorphism, increased plasma Efavirenz concentrations and early neuropsychiatric side effects in South African HIV-infected patients. *AIDS research and therapy.* 2010;7:32.

107. Viljoen M, Karlsson MO, Meyers TM, Gous H, Dandara C, Rheeders M. Influence of CYP2B6 516G>T polymorphism and interoccasion variability (IOV) on the population

pharmacokinetics of efavirenz in HIV-infected South African children. *Eur J Clin Pharmacol.* 2012;68(4):339-347.

108. Swart M, Skelton M, Ren Y, Smith P, Takuva S, Dandara C. High predictive value of CYP2B6 SNPs for steady-state plasma efavirenz levels in South African HIV/AIDS patients. *Pharmacogenet Genomics.* 2013;23(8):415-427.

109. Kwara A, Lartey M, Sagoe KW, Kenu E, Court MH. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. *AIDS.* 2009;23(16):2101-2106.

110. Sarfo FS, Zhang Y, Egan D, et al. Pharmacogenetic associations with plasma efavirenz concentrations and clinical correlates in a retrospective cohort of Ghanaian HIV-infected patients. *The Journal of antimicrobial chemotherapy.* 2013.

111. Mukonzo JK, Roshammar D, Waako P, et al. A novel polymorphism in ABCB1 gene, CYP2B6*6 and sex predict single-dose efavirenz population pharmacokinetics in Ugandans. *British journal of clinical pharmacology.* 2009;68(5):690-699.

112. Ngaimisi E, Habtewold A, Minzi O, et al. Importance of ethnicity, CYP2B6 and ABCB1 genotype for efavirenz pharmacokinetics and treatment outcomes: a parallel-group prospective cohort study in two sub-Saharan Africa populations. *PLoS One.* 2013;8(7):e67946.

113. Ngaimisi E, Mugusi S, Minzi OM, et al. Long-term efavirenz autoinduction and its effect on plasma exposure in HIV patients. *Clinical pharmacology and therapeutics.* 2010;88(5):676-684.

114. Mutwa PR, Fillekes Q, Malgaz M, et al. Mid-dosing interval efavirenz plasma concentrations in HIV-1-infected children in Rwanda: treatment efficacy, tolerability, adherence, and the influence of CYP2B6 polymorphisms. *Journal of acquired immune deficiency syndromes.* 2012;60(4):400-404.

115. Viengchareun S, Caron M, Auclair M, et al. Mitochondrial toxicity of indinavir, stavudine and zidovudine involves multiple cellular targets in white and brown adipocytes. *Antivir Ther.* 2007;12(6):919-929.

116. Wallace DC, Brown MD, Lott MT. Mitochondrial DNA variation in human evolution and disease. *Gene.* 1999;238(1):211-230.

117. Saxena R, de Bakker PI, Singer K, et al. Comprehensive association testing of common mitochondrial DNA variation in metabolic disease. *Am J Hum Genet.* 2006;79(1):54-61.

118. van Oven M, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. *Hum Mutat.* 2009;30(2):E386-394.

119. Kokaze A, Ishikawa M, Matsunaga N, et al. Association of the mitochondrial DNA 5178 A/C polymorphism with serum lipid levels in the Japanese population. *Human genetics.* 2001;109(5):521-525.

120. Lal S, Madhavan M, Heng CK. The association of mitochondrial DNA 5178 C > a polymorphism with plasma lipid levels among three ethnic groups. *Annals of human genetics.* 2005;69(Pt 6):639-644.

121. Park KS, Chan JC, Chuang LM, et al. A mitochondrial DNA variant at position 16189 is associated with type 2 diabetes mellitus in Asians. *Diabetologia.* 2008;51(4):602-608.

122. Dahmani Y, Marcuello A, Diez-Sanchez C, Ruiz-Pesini E, Montoya J, Lopez-Perez MJ. Association of human mitochondrial DNA variants with plasma LDL levels. *Mitochondrion.* 2008;8(3):247-253.

123. Feder J, Ovadia O, Blech I, et al. Parental diabetes status reveals association of mitochondrial DNA haplogroup J1 with type 2 diabetes. *BMC medical genetics.* 2009;10:60.

124. Hart AB, Samuels DC, Hulgan T. The other genome: a systematic review of studies of mitochondrial DNA haplogroups and outcomes of HIV infection and antiretroviral therapy. *AIDS Rev.* 2013;15(4):213-220.

125. Grady BJ, Samuels DC, Robbins GK, et al. Mitochondrial genomics and CD4 T-cell count recovery after antiretroviral therapy initiation in AIDS clinical trials group study 384. *J Acquir Immune Defic Syndr.* 2011;58(4):363-370.

126. Hendrickson SL, Hutcheson HB, Ruiz-Pesini E, et al. Mitochondrial DNA haplogroups influence AIDS progression. *AIDS.* 2008;22(18):2429-2439.

127. Hendrickson SL, Kingsley LA, Ruiz-Pesini E, et al. Mitochondrial DNA haplogroups influence lipoatrophy after highly active antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2009;51(2):111-116.

128. Hulgan T, Haubrich R, Riddler SA, et al. European mitochondrial DNA haplogroups and metabolic changes during antiretroviral therapy in AIDS Clinical Trials Group Study A5142. *AIDS.* 2011;25(1):37-47.

129. De Luca A, Nasi M, Di Giambenedetto S, et al. Mitochondrial DNA haplogroups and incidence of lipodystrophy in HIV-infected patients on long-term antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2012;59(2):113-120.

130. Hulgan T, Tebas P, Canter JA, et al. Hemochromatosis gene polymorphisms, mitochondrial haplogroups, and peripheral lipoatrophy during antiretroviral therapy. *J Infect Dis.* 2008;197(6):858-866.

131. Micheloud D, Berenguer J, Guzman-Fulgencio M, et al. European mitochondrial DNA haplogroups and metabolic disorders in HIV/HCV-coinfected patients on highly active antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2011;58(4):371-378.

132. Hulgan T, Stein JH, Cotter BR, et al. Mitochondrial DNA variation and changes in adiponectin and endothelial function in HIV-infected adults after antiretroviral therapy initiation. *AIDS Res Hum Retroviruses.* 2013;29(10):1293-1299.

133. Canter JA, Haas DW, Kallianpur AR, et al. The mitochondrial pharmacogenomics of haplogroup T: MTND2*LHON4917G and antiretroviral therapy-associated peripheral neuropathy. *Pharmacogenomics J.* 2008;8(1):71-77.

134. Kallianpur AR, Hulgan T, Canter JA, et al. Hemochromatosis (HFE) gene mutations and peripheral neuropathy during antiretroviral therapy. *AIDS.* 2006;20(11):1503-1513.

135. Garcia-Alvarez M, Guzman-Fulgencio M, Berenguer J, et al. European mitochondrial DNA haplogroups and liver fibrosis in HIV and hepatitis C virus coinfected patients. *AIDS.* 2011;25(13):1619-1926.

136. Hendrickson SL, Jabs DA, Van Natta M, Lewis RA, Wallace DC, O'Brien SJ. Mitochondrial haplogroups are associated with risk of neuroretinal disorder in HIV-positive patients. *J Acquir Immune Defic Syndr.* 2010;53(4):451-455.

137. Canter JA, Robbins GK, Selph D, et al. African mitochondrial DNA subhaplogroups and peripheral neuropathy during antiretroviral therapy. *J Infect Dis.* 2010;201(11):1703-1707.

138. Holzinger ER, Hulgan T, Ellis RJ, et al. Mitochondrial DNA variation and HIV-associated sensory neuropathy in CHARTER. *Journal of neurovirology.* 2012;18(6):511-520.

139. Aissani B, Shrestha S, Wiener HW, Tang J, Kaslow RA, Wilson CM. Mitochondrial DNA variation and virologic and immunological HIV outcomes in African Americans. *AIDS.* 2014;28(13):1871-1878.

140. Arenas-Pinto A, Weller I, Ekong R, et al. Common inherited mitochondrial DNA mutations and nucleoside reverse transcriptase inhibitor-induced severe hyperlactataemia in HIV-infected adults: an exploratory study. *Antivir Ther.* 2012;17(2):275-282.

141. Hulgan T, Samuels DC, Bush W, et al. Mitochondrial DNA Haplogroups and Neurocognitive Impairment During HIV Infection. *Clin Infect Dis.* 2015.

142. Kampira E, Kumwenda J, van Oosterhout JJ, Dandara C. Mitochondrial DNA subhaplogroups L0a2 and L2a modify susceptibility to peripheral neuropathy in malawian adults on stavudine containing highly active antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2013;63(5):647-652.

143. Hulgan T, Robbins GK, Kalams SA, et al. T cell activation markers and African mitochondrial DNA haplogroups among non-Hispanic black participants in AIDS clinical trials group study 384. *PLoS One.* 2012;7(8):e43803.

CHAPTER 3

Association of lopinavir concentrations with plasma lipid or glucose concentrations in HIV-infected South Africans: a cross sectional study

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Association of lopinavir concentrations with plasma lipid or glucose concentrations in HIV-infected South Africans: a cross sectional study

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Abstract

Background: Dyslipidaemia and dysglycaemia have been associated with exposure to ritonavir-boosted protease inhibitors. Lopinavir/ritonavir, the most commonly used protease inhibitor in resource-limited settings, often causes dyslipidaemia. There are contradictory data regarding the association between lopinavir concentrations and changes in lipids.

Aim: To investigate associations between plasma lopinavir concentrations and lipid and glucose concentrations in HIV-infected South African adults.

Methods: Participants stable on lopinavir-based antiretroviral therapy were enrolled into a cross-sectional study. After an overnight fast, total cholesterol, triglycerides, and lopinavir concentrations were measured and an oral glucose tolerance test was performed. Regression analyses were used to determine associations between plasma lopinavir concentrations and fasting and 2 hour plasma glucose, fasting cholesterol, and triglycerides concentrations.

Results: There were 84 participants (72 women) with a median age of 36 years. The median blood pressure, body mass index and waist: hip ratio were 108/72 mmHg, 26 kg/m^2 and 0.89 respectively. The median CD4 count was 478 cells/mm^3. Median duration on lopinavir was 18.5 months. The median (interquartile range) lopinavir concentration was 8.0 (5.2 to 12.8) μg/mL. Regression analyses showed no significant association between lopinavir pre-dose concentrations and fasting cholesterol (β-coefficient −0.04 (95% CI −0.07 to 0.00)), triglycerides (β-coefficient −0.01 (95% CI −0.04 to 0.02)), fasting glucose (β-coefficient −0.01 (95% CI −0.04 to 0.02)), or 2-hour glucose concentrations (β-coefficient −0.02 (95% CI −0.09 to 0.06)). Lopinavir concentrations above the median were not associated with presence of dyslipidaemia or dysglycaemia.

Conclusions: There was no association between lopinavir plasma concentrations and plasma lipid and glucose concentrations.

Keywords: Lopinavir, Hypercholesterolaemia, Hypertriglyceridaemia, Impaired glucose metabolism, Antiretroviral therapy, Pharmacokinetics

Introduction

Ritonavir-boosted lopinavir (LPV/r) is a potent protease inhibitor (PI) widely used in a second-line antiretroviral regimens in low and middle-income countries [1]. Treatment with LPV/r has been associated with the development of dyslipidaemia [2,3], which is a known risk factor for cardiovascular complications. The Women's Interagency HIV study showed that dyslipidaemia was more severe in HIV infected women on ritonavir-boosted PI containing antiretroviral therapy (ART), compared with untreated HIV infected women or non-PI based ART [4]. There are conflicting results regarding an association between LPV/r use and the development of insulin resistance and new onset diabetes [5-9]. Furthermore, there are contradictory data as to whether the metabolic toxicity is associated with higher plasma concentrations

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of either lopinavir or ritonavir [10-12]. Widespread long-term PI use may be limited by these metabolic complications. Data regarding an association between plasma lopinavir and plasma glucose concentration are lacking.

The aim of our study was to investigate whether there is an association between plasma lopinavir and plasma lipids and glucose concentrations. We hypothesized that higher plasma lopinavir concentrations would be associated with higher prevalence of dyslipidaemia and dysglycaemia.

Materials and methods

Study design and participants

We conducted a prospective cross sectional study between February 2007 and January 2008. Consecutive ambulatory HIV infected African adults who presented for a routine follow up visit at primary and tertiary level clinics in Cape Town were recruited. Participants were eligible if they were on lopinavir-based therapy for a minimum of six months. Participants with renal or hepatic disease, active opportunistic infection, diabetes, history of diabetes or dyslipidaemia and self-reported non-adherence were excluded. The study was approved by the University of Cape Town research ethics committee.

Clinical and laboratory evaluations

After obtaining informed consent, we instructed the participants to undergo an overnight fast and to note the time of taking the evening dose of lopinavir on the day proceeding the study day. On the study day, participants underwent an oral glucose tolerance tests (OGTT). Blood was drawn at 0 and 120 minutes after ingestion of 75g glucose in 250ml water, into heparinised or sodium fluoride tubes as appropriate, and kept on ice until centrifugation within 4 hours. Plasma lopinavir concentrations and serum fasting glucose, cholesterol, and triglyceride concentrations were determined from the 0 minute samples of the OGTT. The morning doses of ART were given after 2 hour glucose samples were taken.

Lopinavir drug concentrations were analyzed by fully validated methods using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Applied Biosystems MDS Sciex API 4000 tandem mass spectrometer at our ISO17025 compliant and accredited analytical laboratory as previously described [13]. The assay range was 0.05 to 20 μg/mL. Accuracy ranged from 94 to 103%. Any samples with lopinavir concentrations below the limit of quantification (0.05 μg/mL) were fixed to 0.025 μg/mL. Serum glucose and lipid concentrations were determined by standard methods using the ACE Alera Clinical Chemistry System (Alfa Wassermann Diagnostic Technologies, Woerden, Netherlands). Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined according to the American Diabetes Association criteria [14]. Hypercholesterolaemia and hypertriglyceridaemia were defined according to the NCEP III criteria [15].

We reviewed medical records to determine duration on antiretroviral therapy, viral load and CD4 counts. Self reported adherence was determined using a validated 4-day adherence questionnaire administered by trained field workers [16]. Basic anthropometric measurements were measured by a biokineticist.

Pharmacokinetic and statistical analysis

The plasma lopinavir concentrations were obtained from 0 minute of the OGTT. For data points below the limit of quantification (0.05 μg/mL), the lopinavir concentrations were fixed to 0.025 μg/mL. The associations between lopinavir concentrations and glucose, triglycerides, and cholesterol were determined using univariate linear regression and multivariate analyses. Logistic regression was used to determine associations between lopinavir concentrations above the median and metabolic complications (hypercholesterolaemia, hypertriglyceridaemia and dysglycaemia).

Sample size calculation

A previous study found a positive correlation (rho) of 0.32 between lopinavir trough levels and triglyceride levels [10]. When the sample size is 50, the linear regression test of rho=0 (alpha= 0.050 two-sided) for normally distributed triglyceride concentrations, will have 80% power to detect a rho of 0.375.

Results

Of 93 participants enrolled for the study, nine were excluded due to missing anthropometric and metabolic data or inaccurate dose-sampling time. The baseline characteristics of the 84 participants included in the analysis are in Table 1. The majority were women, reflecting the epidemic seen in our clinical practice. All participants reported 100% adherence. The prevalence of hypercholesterolaemia, hypertriglyceridaemia, impaired fasting glucose, impaired glucose tolerance, and diabetes were 29%, 29%, 25%, 14% and 4% respectively. The plasma LPV concentrations are shown in Figure 1. The median (IQR) time after dose was 13.2 (12.6 to 14.1) hours. There were ten participants whose lopinavir concentrations were below 1 μg/mL (12%). Of these, 4 were virologically suppressed, 4 had virologic failure, and 2 had missing viral load data. As there was a significant association between time after dose and lopinavir concentrations, the time after dose was included in the multivariate regression analyses investigating the association between lopinavir concentrations and metabolic parameters. There were no significant associations between lopinavir concentrations and lipid and glucose

Table 1 Study population characteristics, N=84

Variable	Median (IQR) or n/N(%)
Age (years)	36 (32–42)
Female n/N (%)	72/84 (86)
Race n/N (%)	
Black	84/84 (100)
Weight (kg)	69 (60–82)
Body mass index (kg/m^2)	26 (23–32)
Waist: hip ratio	0.88 (0.82-0.94)
Skin fold thickness (mm)	
Triceps	17 (11–25)
Abdomen	24 (17–40)
Thigh	30 (17–46)
Calf	16 (9–21)
Blood pressure mmHg	108/72 (102/66-119/77)
CD4 count (cells/ mm^3)	
Pre-ART	103 (37–140)
Current	468 (291–623)
Current viral load	
Proportion with <400 copies/mL (%)*	64/74 (86)
Duration on lopinavir (months)	19 (9–29)
Concurrent ART n/N (%)	
Zidovudine/didanosine	51/84 (61)
Zidovudine/lamivudine	17/84 (20)
Stavudine/lamivudine	10/84 (12)
Efavirenz	4/84 (5)
Nevirapine	2/84 (2)
Metabolic parameters (mmol/L)	
Fasting cholesterol	4.3 (3.7 to 5.3)
Fasting triglycerides	1.3 (0.9 to 1.8)
Fasting glucose	5.2 (4.7 to 5.7)
2 hour glucose	6.3 (5.4 to 8.1)

* 10 participants had missing current viral load data.

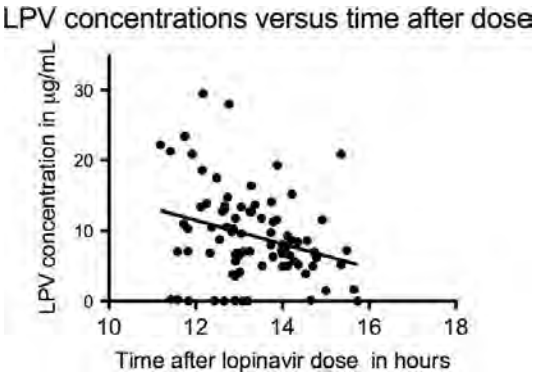

Figure 1 Scatter plot of lopinavir concentrations (µg/mL) and the time after the last lopinavir dose (hours). The black dots denote individual lopinavir concentrations plotted against the time after the last unobserved lopinavir dose (dose to sampling time) from 84 participants. The regression line shows negative correlation between the lopinavir concentrations and the dose to sampling time (Spearman rho (95%CI)= −0.28(−0.47 to −0.06), p-value 0.01).

concentrations on simple regression analyses, or after adjusting for age, sex, time after dose, and duration on lopinavir (Table 2). The lack of association between lopinavir concentrations and the metabolic parameters persisted when the 10 participants with lopinavir concentrations below 1 µg/mL (as they were probably non-adherent) were excluded (data not shown). Sixty one percent of participants were on zidovudine and didanosine combination (Table 1), as recommended by the South African antiretroviral guidelines at the time. However, the different NRTI backbone was not associated with any of the outcomes evaluated. For example, for triglycerides, in the participants of the same age, sex, duration on lopinavir, and with same lopinavir concentration taken at the same time after dose, there was no significant difference between those who were on zidovudine/didanosine combination compared to other NRTI combinations [mean difference −0.22(−0.65 to 0.21) mmol/L, p value =0.31]. Similarly, there was no association with NRTI backbone and cholesterol if there all parameters remained the same [mean difference −0.18(−0.73 to 0.37) mmol/L, p value =0.51]. There was no association between lopinavir concentrations above the median (8 µg/mL) and dyslipidaemia or dysglycaemia (see Figure 2).

Discussion

To our knowledge, this is the first study to investigate the association between lopinavir concentrations and serum glucose concentrations, and the first to investigate associations between lopinavir concentrations and serum lipids in a South African population. Despite a high prevalence of dyslipidaemia (29%) and dysglycaemia (42%) in 84 black South African HIV-infected adults treated with ritonavir-boosted lopinavir for a median duration of 19 months, we found no association between plasma lopinavir concentrations and lipid or glucose concentrations. There was also no significant association between the lopinavir concentrations above the median, and hypercholesterolaemia, hypertriglyceridaemia or dysglycaemia.

We found the median lopinavir concentration was 8 µg/mL, which is higher than reported elsewhere [17,18], but comparable to the trough concentrations after observed doses in a study conducted by our group from the same community [19]. Lopinavir pharmacokinetics

Table 2 Association between lopinavir trough concentrations and lipid and glucose concentrations

	Univariate analyses		Multivariate analyses	
Variable	**Beta coefficient (95%CI)**	**P value**	**Beta coefficient (95% CI)**	**P value**
Total cholesterol	−0.04 (−0.07 to 0.00)	0.07	−0.02 (−0.06 to 0.01)	0.21
Triglycerides	−0.01 (−0.04 to 0.02)	0.53	−0.00 (−0.03 to 0.02)	0.86
Fasting glucose	−0.01 (−0.04 to 0.02)	0.44	−0.00 (−0.03 to 0.02)	0.78
2 hour glucose	−0.02 (−0.09 to 0.06)	0.64	0.00 (−0.05 to 0.06)	0.85

Adjusted for time after dose, age, sex and duration on lopinavir. In the multivariate model for 2 hour glucose, time after dose was an independent predictor [β-coefficient 0.56 (95% CI 0.23 to 0.88), p= 0.001].

demonstrate considerable interindividual variability, which may affect treatment outcomes. At least part of this variability may be explained by host genetic factors. Associations between human genetic variants and lopinavir exposure are incompletely understood and need to be explored.

The lack of an association between lopinavir concentrations and lipid or glucose concentrations that we found can be explained as follows: First, like all protease inhibitors, lopinavir exerts its antiviral activity intracellularly, and the plasma and intracellular half-lives are different [20]. However, the positive correlation between lopinavir plasma and intracellular concentrations reported at 4 weeks was not sustained at 24 weeks of treatment [20]. More importantly, the mechanism of toxicity associated with protease inhibitor use, including lopinavir, is still poorly understood, but is thought to be related to interference with some cellular endogenous processes. For example, lopinavir binds to lipoprotein receptor related protein (LPR), impairing hepatic chylomicron uptake and triglyceride clearance by LPR –lipoprotein lipase complex, causing hyperlipidaemia [21]. In susceptible individuals, the resulting hyperlipidaemia may induce diabetes [21]. Second, clinical manifestations of LPV/r toxicity are also influenced by host susceptibility such as age, sex, weight, race, advanced HIV disease, concomitant ART, higher baseline triglyceride or cholesterol concentrations, and genetic susceptibility [2,22,23]. Finally, we hypothesize that the if the association between plasma lopinavir concentrations and toxicity exists, it exists in much lower concentrations, as the dose–response curve is likely to be flat at the concentrations found in our study. Therefore, the influence of lopinavir plasma concentrations on lipids at therapeutic doses, is likely to be small and larger studies are needed to detect the small differences. Furthermore, recent data suggests that lopinavir does not impair insulin sensitivity [7,8], and therefore, the lack of an association between lopinavir and glucose concentrations is not surprising.

Our findings are in contrast with findings from a small study conducted in 19 patients, which reported that

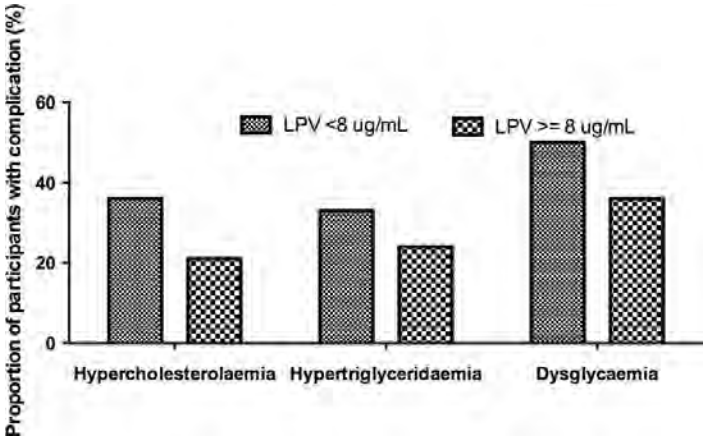

Figure 2 Proportion of participants with dyslipidaemia and dysglycaemia categorized by the lopinavir concentration below or above the median (8μg/mL). The bar graphs show the proportion of participants (%) who had hypercholesterolaemia, hypertriglyceridaemia and dysglycaemia in 42 participants with lopinavir below the median (fine checked bars) and in 42 participants with lopinavir concentrations equal to, or above the median (course checked bars). There was no association between the lopinavir concentrations above the median and the metabolic complications. The odds ratios for lopinavir above the median and the complications were: hypercholesterolaemia OR (95%CI): 0.49 (0.19 to 1.30); hypertriglyceridaemia OR (95%CI): 0.56 (0.29 to 1.33); dysglycaemia OR (95 CI): 0.63 (0.24 to 1.63).

lopinavir trough concentrations were higher in three patients experiencing grade 3 or 4 lipid elevations [11]. A second larger study (n=126) found that patients with fasting triglyceride concentrations above the median had higher lopinavir trough concentrations, but no correlation was found between lopinavir and cholesterol concentrations [10]. Four other studies reported findings similar to ours, with no association between lopinavir and lipid concentrations [12,17,24,25]. The older protease inhibitor indinavir is known to cause diabetes [5]. However, after 12 months of treatment with lopinavir, none of the 73 patients included in another study developed diabetes [6]. Studies in healthy volunteers have shown that insulin sensitivity wasn't altered by lopinavir in healthy HIV negative men [7,8]. However, a single dose study in healthy volunteers showed that lopinavir could inhibit glucose uptake acutely [9]. Data regarding the association between lopinavir concentrations and glucose metabolism are lacking.

Our study has several limitations. First, it is a cross sectional study and we therefore cannot compare lipid or glucose concentrations prior to lopinavir treatment. Second, patients with known diabetes or dyslipidaemia were excluded from the study, and it is possible that lopinavir may have exacerbated a pre-existing metabolic defect. Third, our sample size is relatively small, however, it is larger than most of the previous studies that have investigated this association [11,17,19]. We aimed to examine 50 participants to detect a correlation of 0.375. Our analyses used simple and multivariate regression analyses with various predictors, therefore we continued to slowly recruit eligible participants until the end of the study. Fourth, we investigated associations with pre-dose lopinavir concentrations and metabolic parameters. The pharmacokinetic parameters area under the curve or average steady state would be a better measure of overall drug exposure. Lastly, the last dose of lopinavir was not observed. To minimize recall bias, participants were requested to record the time of last dose on the appointment card for the day before pharmacokinetic sampling.

In conclusion, we did not find an association between lopinavir concentrations and lipid and glucose concentrations. Larger prospective studies are needed to establish whether an association exists between lopinavir concentrations and increasing lipids or glucose metabolism changes.

Competing interests
The authors declare that they have no competing interests.

Authors' contributions
PZS participated in the study design, acquisition of data, data analysis and interpretation and drafted the manuscript. HMM participated in the study design, data interpretation, and critically revised the manuscript. PJS performed the analysis of the samples and helped to draft the manuscript. JAD participated in the study design, acquisition of data, and critically revised the manuscript. NSL participated in study design and critically revised the manuscript. GM conceived of the study, participated in study design, data interpretation and critically revised the manuscript. All authors read and approved the final manuscript.

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These data were presented in part at the 12th International Workshop on Adverse Drug Reactions and Co-morbidities in HIV, London, UK, 5 November 2010 (Abstract ADRLH-48). The abstract was also published in Antiviral Therapy 2010; 15 Suppl 4: A61 (abstract no P32).

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References
1. World Health Organization: **Rapid advice antiretroviral therapy for HIV infection in adults and adolescents**. *World Health Organization*; 2009. downloaded from http://www.who.int/hiv/pub/arv/rapid_advice_art.pdf on 03 August 2011.
2. De Wit S, Sabin CA, Weber R, Worm SW, Reiss P, Cazanave C, El-Sadr C, Monforte W, Fontas A, Law E, Friis-Møller MG, Phillips N: **Incidence and risk factors for new onset diabetes in HIV-infected patients. The data collection on adverse events of anti-HIV drugs (D: A: D) study.** *Diabetes Care* 2008, **31:**1224–1229.
3. Montes ML, Pulido F, Barros C, Condes E, Rubio R, Cepeda C, Dronda F, Antela A, Sanz J, Navas E, Miralles P, Berenguer J, Pérez S, Zapata A, González-García JJ, Peña JM, Vázquez JJ, Arribas JR: **Lipid disorders in antiretroviral-naive patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors.** *JAC* 2005, **55:**800–804.
4. Anastos K, Lu D, Shi Q, Tien PC, Kaplan RC, Hessol NA, Cole S, Vigen C, Cohen M, Young M, Justman J: **Association of serum lipid levels with HIV serostatus, specific antiretroviral agents, and treatment regimens.** *J Acquir Immune Defic Syndr* 2007, **45:**34–42.
5. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA: **Diagnosis, prediction and natural course of HIV-1 protease-inhibitor associated lipodystrophy hyperlipidaemia, and diabetes mellitus: a cohort study.** *Lancet* 1999, **353:**2093–2099.
6. Galindo MJ, Verdejo J, Gonzalez-Mun M, Ferrer A, Polo R: **Metabolic changes in protease inhibitor-naïve patients with lopinavir/ritonavir.** *J Acquir Immune Defic Syndr* 2008, **48:**628–629.
7. Dube' MP, Shen C, Greenwald M, Mather KJ: **No impairment of endothelial function or insulin sensitivity with 4 weeks of the HIV protease inhibitors atazanavir or lopinavir-ritonavir in healthy subjects without HIV infection: A placebo-controlled trial.** *Clinical Infectious Disease* 2008, **47:**567–574.
8. Pao VY, Lee GA, Taylor S, Aweeka FT, Schwarz JM, Mulligan K, Schambelan M, Grunfeld C: **The protease inhibitor combination lopinavir/ritonavir does not decrease insulin secretion in healthy, HIV-seronegative volunteers.** *AIDS* 2010, **24:**265–270.
9. Lee GA, Lo JC, Aweeka F, Schwarz JM, Mulligan K, Schambelan M, Grunfeld C: **Single-dose lopinavir acutely inhibits insulin-mediated glucose disposal in healthy volunteers.** *Clin Infect Dis* 2006, **43:**658–660.

10. de González Requena D, Blanco F, Garcia-Benayas T, *et al:* **Correlation between lopinavir plasma levels and lipid abnormalities in patients taking Lopinavir/ritonavir.** *AIDS Patient Care STDS* 2003, **17:**443–445.
11. Gutiérrez F, Padilla S, Navarro A, Masiá M, Hernández I, Ramos J, Esteban A, Martin-Hidalgo A: **Lopinavir plasma concentrations and changes in lipid levels during salvage therapy with lopinavir/ritonavir-containing regimens.** *J Acquir Immune Defic Syndr* 2003, **33:**594–600.
12. León A, Martinez E, Sarasa M, López Y, Mallolas J, De Lazzari E, Laguno M, Milincovic A, Blanco JL, Larrousse M, Lonca M, Gatell JM: **Impact of steady-state lopinavir plasma levels on plasma lipids and body composition after 24 weeks of Lopinavir containing therapy free of thymidine analogues.** *J Antimicrob Chemother* 2007, **60:**824–830.
13. Ren Y, Nuttall JJ, Egbers C, Eley BS, Meyers TM, Smith PJ, Maartens G, McIlleron HM: **Effect of rifampicin on lopinavir pharmacokinetics in HIV-infected children with tuberculosis.** *J Acquir Immune Defic Syndr* 2008, **47:**566–569.
14. American Diabetes Association: **Diagnosis and classification of diabetes mellitus.** *Diabetes Care* 2008, **31:**S55–S60.
15. Grundy SM, Cleeman JI, Merz CN, Brewer HB Jr, Clark LT, Hunninghake DB, Pasternak RC, Smith SC Jr, Stone NJ: **Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines.** *Circulation* 2004, **110:**227–239.
16. ACTG adherence follow-up questionnaire; 2006. http://www.caps.ucsf.edu/tools/surveys/pdf/2098.4188.pdf, on the 22 December 2006.
17. Hurst M, Faulds D: **Lopinavir.** *Drugs* 2000, **60:**1371–1379.
18. Bierman WF, van Vonderen MG, Veldkamp AI, Burger DM, Danner SA, Reiss P, van Agtmael MA: **The lopinavir/ritonavir-associated rise in lipids is not related to lopinavir or ritonavir plasma concentration.** *Antivir Ther* 2011, **16:**647–655.
19. Decloedt EH, McIlleron H, Smith P, Merry C, Orrell C, Maartens G: **Pharmacokinetics of lopinavir in HIV-infected adults receiving rifampicin with adjusted doses of lopinavir-ritonavir tablets.** *AAC* 2011, **55:**3195–3200.
20. Bazzoli C, Jullien V, Le Tiec C, Rey E, Taburet AM: **Intracellular pharmacokinetics of antiretroviral drugs in HIV infected patients, and their correlation with drug action.** *Clin Pharmacokinet* 2010, **49:**17–45.
21. Carr A, Samaras K, Chisholm DJ, Cooper D: **Pathogenesis of HIV-1-protease inhibitor associated peripheral lipodystrophy, hyperlipidaemia and insulin resistance.** *Lancet* 1998, **351:**1881–1883.
22. Bongiovanni M, Bini T, Cicconi P, Landonio S, Meraviglia P, Testa L, Di Biagio A, Chiesa E, Tordato F, Biasi P, Adorni F, Monforte AD: **Predictive factors of hyperlipidemia in HIV-infected subjects receiving lopinavir/ritonavir.** *AIDS Res Hum Retroviruses* 2006, **22:**132–138.
23. Tarr P, Telenti A: **Toxigenetics of antiretroviral therapy: genetic factors that contribute to metabolic complications.** *Antivir Ther* 2007, **12:**999–1013.
24. Rhee MS, Hellinger JA, Sheble-Hall S, Cohen CJ, Greenblatt DJ: **Relationship between plasma protease inhibitor concentrations and lipid elevations in HIV patients on a double-boosted protease inhibitor regimen (saquinavir/lopinavir/ritonavir).** *J Clin Pharmacol* 2010, **50:**392–400.
25. Ter Hostede HJM, Koopmans PP, Burger DM, Sprenger HG, Napel CT, Vriesendorp V, Richter C: **Lopinavir plasma concentrations and serum lipids in therapy naïve HIV-patients: A sub- analysis of the FREE study.** *Pharmacology & Pharmacy* 2012, **3:**90–96.

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CHAPTER 4

Mitochondrial genomics and antiretroviral therapy-associated metabolic complications in HIV-infected Black South Africans: a pilot study

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Mitochondrial Genomics and Antiretroviral Therapy-Associated Metabolic Complications in HIV-Infected Black South Africans: A Pilot Study

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Abstract

Studies suggest that mitochondrial DNA (mtDNA) haplogroups are associated with antiretroviral therapy (ART)-related metabolic complications and distal sensory polyneuropathy (DSP), but there have been few studies in persons of African descent. We explored such associations in South African adults. Clinical and laboratory data and DNA specimens from a cross-sectional study were used. Sequencing and Phylotree determined African mtDNA subhaplogroups. Wilcoxon and regression analyses determined associations between mtDNA subhaplogroups and ART-related complications. The 171 participants represented six major haplogroups: L0 ($n=78$), L1 ($n=3$), L2 ($n=30$), L3 ($n=53$), L4 ($n=1$), and L5 ($n=6$). Analyses were restricted to 161 participants representing L0, L2, and L3: 78% were female; the median age was 36 years. All had been exposed to thymidine analogues, 42% were on lopinavir/ritonavir (lopinavir/r), and 58% were on either efavirenz or nevirapine. Median (IQR) ART duration was 22 (14–36) months. Median fasting triglycerides were 1.60 (1.13–1.75) and 1.04 (0.83–1.45) mmol/liter among L3e1 ($n=22$) and other subhaplogroups, respectively ($p=0.003$). Subhaplogroup L3e1 [adjusted OR (aOR) 3.16 (95% CI: 1.11–8.96); $p=0.03$] and exposure to lopinavir/r [aOR 2.98 (95% CI: 1.02–8.96); $p=0.05$] were independently associated with hypertriglyceridemia, after adjusting for age, sex, and ART duration. There were no significant associations between mtDNA haplogroups and cholesterol, dysglycemia, hyperlactatemia, or lipoatrophy, or DSP. Subhaplogroup L3e1 and lopinavir/r exposure were independently associated with hypertriglyceridemia in black South Africans on ART. This is the first report to link an African mtDNA variant with hypertriglyceridemia. If replicated, these findings may provide new insights into host factors affecting metabolic complications.

Introduction

Exposure to antiretroviral therapy (ART) has been associated with metabolic adverse effects such as dyslipidemia, insulin resistance, dysglycemia, central fat accumulation, peripheral fat loss (lipoatrophy), and peripheral neuropathy.[1,2] Many of these adverse effects are thought to be related to nucleoside reverse transcriptase inhibitor (NRTI)-related mitochondrial toxicity, at least in part due to inhibition of mitochondrial DNA (mtDNA) gamma polymerase and subsequent mitochondrial dysfunction.[2] Other antiretroviral classes have also been associated with mitochondrial dysfunction.[3,4]

Mitochondrial DNA is distinct from nuclear DNA and codes for 13 polypeptides essential for oxidative phosphorylation.[5] Mitochondrial DNA exhibits abundant genetic variation across the 16.6 kb mitochondrial genome. Human mtDNA sequences have diverged over approximately the last 150,000 years due to natural selection and human migration, resulting in distinct patterns of single nucleotide polymorphisms (SNPs), called haplogroups.[6] Evidence for associations between mtDNA haplogroups and outcomes in HIV-infected participants has been reported for CD4 count recovery,[7] AIDS progression,[8] and ART-related complications.[9,10,11,12,13,14,15] Studies in HIV-uninfected populations (primarily European and Asian) have reported associations between mtDNA

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and metabolic complications including dyslipidemia and diabetes.[16,17,18,19,20]

The majority of HIV-infected persons worldwide reside in sub-Saharan Africa,[21] but this region has been underrepresented in genetic studies. Studies in African-Americans showed associations between African mtDNA subhaplogroup L1c and peripheral neuropathy,[15] and between haplogroup L2 and CD4 T cell recovery.[7] To date, no published studies have investigated associations between African mtDNA haplogroups and important metabolic complications including lipoatrophy, dysglycemia, or dyslipidemia. In this study, we report the prevalence of mtDNA haplogroups in a South African population enrolled in an ART program, and explore associations between African mtDNA and ART-associated complications including metabolic complications and distal sensory polyneuropathy (DSP). Because ART-associated adverse effects in HIV-infected persons, and metabolic diseases in general, are believed to result in part from mitochondrial dysfunction, we hypothesized that mtDNA variation would be associated with susceptibility to ART-associated lipoatrophy, dysglycemia, hypertriglyceridemia, and/or distal peripheral neuropathy in this South African population.

Materials and Methods

Study design and participants

This analysis was conducted by retrospectively analyzing data and specimens that had been collected during a prospective, cross-sectional study of the prevalence of metabolic and neuropathy complications of ART.[22,23] Participants were ambulatory HIV-infected African black adults who presented for a routine follow-up visit at public-sector antiretroviral clinics in Cape Town. Participants were recruited by convenience sampling between February 2007 and September 2008. They were eligible if they were on ART therapy for at least 6 months, with no renal or hepatic disease, no active opportunistic infections, or no known history of diabetes or dyslipidemia. Participants on lipid or glucose-lowering therapy were also excluded. The University of Cape Town research ethics committee approved the primary study and all participants who signed informed consent for the genetic study and had clinical and laboratory evaluations and/or whole body dual-energy X-ray absorptiometry (DEXA) were included in this analysis. The Vanderbilt University Institutional Review Board approved the use of de-identified DNA and clinical data for these analyses.

Clinical and laboratory evaluations

Participants fasted overnight and underwent an oral glucose tolerance test (OGTT). Impaired fasting glucose (IFG), impaired glucose tolerance (IGT), and diabetes were defined according to the American Diabetes Association criteria.[24] Fasting triglyceride concentrations were determined at the start of the OGTT and were measured at the National Health Laboratory Services. Hypertriglyceridemia was defined according to NCEP III criteria.[25] Point-of-care measurement of lactate was done before glucose loading using the Accutrend lactate meter (Roche, Basel, Switzerland). Hyperlactatemia was defined as a lactate concentration ≥ 2.5 mmol/liter.[26] For the present study, DSP was defined as the presence of at least two of the following signs: reduced or absent reflexes and impaired vibratory or pinprick sensation with or without neuropathic symptoms (pain, paresthesia, or numbness). A DSP diagnosis was considered only if signs and symptoms were bilateral, of symmetrical onset, and present for at least 2 weeks. Limb fat was measured by DEXA scan and percentage of limb fat was used to determine lipoatrophy, which was defined as percentage limb fat below the median stratified by sex. We reviewed medical records to determine duration on antiretroviral therapy, current $CD4^+$ lymphocyte counts, and viral load within 3 months of the study visit, where available.

Mitochondrial DNA isolation and sequencing

Chromosomal and mtDNA were isolated from buffy coats using Maxwell16® DNA Purification Kits (Promega Corporation, Madison, WI). Full mitochondrial DNA sequencing was performed using the GeneChip Human Resequencing Array v2.0 (Affymetrix Inc., Santa Clara, CA). The full sequence data were used to assign haplogroups and subhaplogroups based on PhyloTree.[27]

Statistical analysis

Kruskal–Wallis and Wilcoxon rank-sum tests were used to compare medians between haplogroups. Linear regression models were used to compare continuous outcomes between subhaplogroups. For analysis of binary outcomes, a case-control design was used. The Pearson Chi-squared test was used to compare proportions of subjects with dichotomous metabolic outcomes. Logistic regression models were fitted to assess the association between subhaplogroups and dysglycemia, DSP, hyperlactatemia, hypertriglyceridemia, and lipoatrophy. Age, sex, lopinavir exposure, and duration on ART were included in all multivariate regression models. Age and sex were included a priori as potential confounders. Lopinavir was included as the potential confounder as it had been associated with metabolic complications. The duration on ART was included in all multivariate models as it differed between the major haplogroups. Subhaplogroups with fewer than 10 participants were excluded from analyses. Analyses were performed using Stata release 11 (StataCorp LP, College Station TX). All tests were two-sided, and a p value < 0.05 was considered significant. As this was an exploratory study, we did not formally correct for multiple comparisons.

Results

Participant characteristics and mitochondrial haplogroups

A total of 219 participants provided DNA for analysis, and mitochondrial sequencing was successful in 171 (78%). The primary reason for lack of sequencing data was insufficient or poor quality DNA. Figure 1 shows the flow chart of participants in this study. We restricted our analyses to the 161 participants who belonged to the L0 ($n = 78$), L2 (30), and L3 (53) major haplogroups (Table 1) because the numbers in the other haplogroups were small [L4 (1), L1 (3), and L5 (6)]. Most participants (78%) were female, which reflects the population treated in the referring clinics. Current ART regimens contained efavirenz, nevirapine, and lopinavir/ritonavir in 33%, 25%, and 42%, respectively. Consistent with South African HIV treatment guidelines at the time, most participants on a

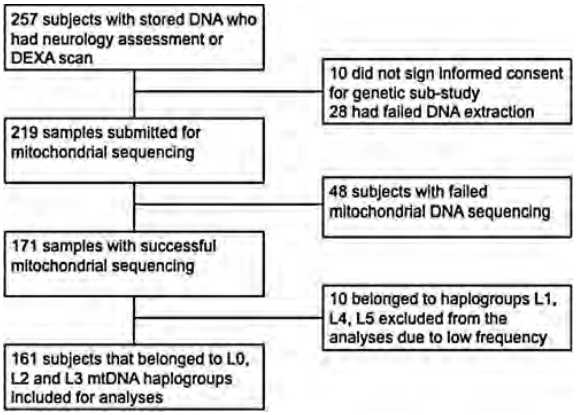

FIG. 1. Flow chart of the study population.

first-line regimen were treated with stavudine/lamivudine (66%) or zidovudine/lamivudine (34%) combinations as the NRTI backbone, while most of those on second-line regimens were treated with zidovudine/didanosine (58%) or zidovudine/lamivudine (24%) combinations. Duration on ART differed across haplogroups (Kruskal–Wallis $p = 0.02$; Table 1), with the L0 major haplogroup having significantly shorter ART duration ($p = 0.01$), and the L3 major haplogroup having longer ART duration compared to the others ($p = 0.01$). However, the duration on lopinavir/ritonavir did not differ among the major haplogroups (Kruskal–Wallis $p = 0.88$; Table 1). The L2 major haplogroup had fewer females than other haplogroups ($p = 0.04$). Otherwise, baseline characteristics did not differ significantly by major mtDNA haplogroup.

Metabolic complications and mitochondrial haplogroups

Median [interquartile range (IQR)] lipid values in this cohort were total cholesterol 4.33 (3.79–5.28) mmol/liter, LDL 2.71 (2.20–3.20) mmol/liter, HDL 1.03 (0.81–1.29) mmol/liter, and triglycerides 1.11 (0.85–1.59) mmol/liter. Simple regression analyses showed no statistically significant associations between the metabolic parameters and the three haplogroups ($p > 0.05$ for each analysis, Table 2).

Metabolic complications were common in this cohort. Hypercholesterolemia was present in 31%, hypertriglyceridemia in 18%, dysglycemia in 34%, and hyperlactatemia in 28%. Table 3 shows unadjusted and adjusted odds ratios for metabolic complications. In these analyses, there was a statistically significant association between the L3 haplogroup and hypertriglyceridemia [unadjusted odds ratio (OR) 2.58 (95% CI 1.14–5.84), $p = 0.02$; Table 3]. This association remained significant after adjusting for age, sex, current lopinavir-based ART, and overall ART duration [adjusted odds ratio (aOR) 2.44(95% CI 1.03–5.77), $p = 0.04$]. As expected, lopinavir/r use was also independently associated with hypertriglyceridemia [aOR 3.55 (95% CI 1.21–10.39), $p = 0.02$].

Every mitochondrial haplogroup is further divided into subhaplogroups.[27] A weak association with a haplogroup might be driven by a stronger association with a subhaplogroup. Because of the availability of full mtDNA sequence data in this study, we were able to explore whether a subhaplogroup within L3 was associated with hypertriglyceridemia. The frequencies of the L3 subhaplogroups in the study population were L3e1 (23), L3e2 (16), L3d1 (4), L3d3 (3), L3e4 (3), L3f1 (2), L3b1 (1), and L3e3 (1). Based on the infrequency of non-L3e1 and non-L3e2 subhaplogroups, we chose to analyze subhaplogroups L3e1 and L3e2 separately, and to combine the remaining participants as "other L3 subhaplogroups."

Figure 2 shows the triglyceride concentrations (Fig. 2A) and hypertriglyceridemia (Fig. 2B) in the L3e1 subhaplogroup, other L3 subhaplogroups, and the haplogroups L0 and L2. The triglyceride concentrations differed significantly between the mtDNA subhaplogroups (Kruskal–Wallis $p = 0.047$). The L3e1 subhaplogroup had significantly higher triglyceride concentrations compared to other subhaplogroups combined (Wilcoxon $p = 0.003$; Fig. 2A). Linear regression showed that mean difference in triglyceride concentrations between the L3e1 subhaplogroup and other subhaplogroups combined was 0.40 mmol/liter [(95% CI 0.06–0.75 mmol/liter), $p = 0.02$]. After adjusting for age, sex, lopinavir/ritonavir-based ART, and ART duration; the association was no longer statistically significant [mean difference 0.35 (95% CI −0.01–0.72) mmol/liter, $p = 0.06$]. The prevalence of hypertriglyceridemia in the L0 and L2 haplogroups and L3 subhaplogroups was L0 (13%) and L2 (17%), L3e1 (41%), L3e2 (19%), and other L3 subhaplogroups (23%). When comparing L3e1 to other subhaplogroups combined, the prevalence of hypertriglyceridemia was 41% and 15%, respectively ($p = 0.004$; Fig. 2B). By logistic regression, there was a statistically significant association between the L3e1 subhaplogroup and hypertriglyceridemia [unadjusted OR 3.82 (95% CI 1.45–10.10), $p = 0.007$]. When adjusted for age, sex, current lopinavir treatment, and ART duration, there remained a significant association with the L3e1 subhaplogroup [aOR 3.16 (95% CI 1.11–8.96), $p = 0.03$]. Lopinavir treatment was also independently associated with hypertriglyceridemia [aOR 2.97 (95% CI 1.02–8.69), $p = 0.05$]. Figure 3 summarizes various multivariate models for the metabolic complications.

There were no other significant associations between haplogroups and metabolic complications other than hypertriglyceridemia. In multivariate analyses for each haplogroup, increasing age was an independent predictor of dysglycemia after adjusting for mtDNA haplogroup, sex, current lopinavir/r treatment, and duration on ART (p for age in each model < 0.05; Fig. 3C).

Peripheral lipoatrophy and mitochondrial haplogroups

Overall median (IQR) percentage limb fat by DEXA was 17 (12–21)% (Table 1). Percentage limb fat differed by sex, with males having a median (IQR) of 8 (6–10)% and females having a median (IQR) of 18 (15–22)%. Initial unadjusted analyses demonstrated significantly lower percentage limb fat in the L2 haplogroup compared with the other haplogroups ($p = 0.04$). However, the L2 haplogroup also had a lower percentage of females than the other haplogroups ($p = 0.04$). In view of these findings, peripheral lipoatrophy was defined as percentage limb fat below the median in sex-stratified populations (male $n = 30$; female $n = 101$). Logistic regression analyses stratified by sex showed no associations between L2 haplogroup and peripheral lipoatrophy by this definition [males: unadjusted OR 1.33 (95% CI 0.30–5.91), $p = 0.71$; females: unadjusted OR 1.17 (95% CI 0.41–3.36), $p = 0.71$]. Adjusting for age and ART

Table 1. Study Population Characteristics Including Metabolic and Neuropathic Data, by Major Haplogroups

	Study population submitted for sequencing		*Major haplogroups analyzed*		
Variable	*All subjects* N = *219*	*Subjects L0, L2, L3* N = *161*	*L0* N = *78*	*L2* N = *30*	*L3* N = *53*
Age (years)	36 (31–42)	36 (32–42)	35 (31–40)	36 (31–41)	37 (32–44)
Female *n/N* (%)[a]	179/219 (82)	125/161 (78)	63/78 (80)	19/30 (63)	43/53 (81)
BMI (kg/m^2)	26 (23–31) *N* = 216	25 (23–28) *N* = 158	25 (23–29) *N* = 77	24 (21–28) *N* = 30	27 (24–34) *N* = 51
CD4 count at start of treatment (cells/mm^3)	97 (48–151) *N* = 199	97 (54–153) *N* = 146	93 (40–155) *N* = 69	79 (33–130) *N* = 28	113 (75–179) *N* = 49
CD4 count at study enrollment (cells/mm^3)	385 (247–520) *N* = 217	382 (247–518) *N* = 160	346 (238–510) *N* = 77	349 (201–518) *N* = 30	447 (288–524) *N* = 53
Viral load (VL) $\log_{10}$					
VL before ART (copies/ml)	4.64 (3.68–5.26) *N* = 100	4.97 (4.49–5.45) *N* = 75	4.98 (4.56–5.49) *N* = 30	4.98 (4.55–5.38) *N* = 14	4.85 (4.48–5.33) *N* = 31
VL < 400 at study enrollment *n/N* (%)	142/163 (87)	109/127 (86)	50/58 (86)	23/27 (85)	36/42 (85)
Blood pressure (mm Hg)	109/73 (101/66–119/80)	107/73 (101/57–119/81)	108/71 (101/63–119/77)	109/75 (100/64–120/79)	107/73 (102/67–120/81)
Waist:hip ratio	0.88 (0.84–0.94)	0.88 (0.83–0.94)	0.86 (0.82–0.94)	0.88 (0.84–0.93)	0.89 (0.85–0.94)
NRTI backbone *n/N* (%)					
Stavudine/lamivudine	97/218 (45)	69/160 (43)	41/77 (53)	8/30 (27)	20/53 (38)
Zidovudine/lamivudine	66/218 (30)	46/160 (28)	19/77 (25)	10/30 (33)	17/53 (32)
Zidovudine/didanosine	46/218 (21)	38/160 (23)	12/77 (16)	12/30 (40)	14/53 (26)
Other	9/218 (4)	7/160 (5)	5/77 (7)	0/30 (0)	2/53 (4)
NNRTI/PI *n/N* (%)					
Efavirenz	82/219 (37)	54/161 (33)	27/78 (35)	6/30 (20)	21/53 (40)
Nevirapine	57/219 (26)	40/161 (25)	24/78 (30)	9/30 (30)	7/53 (13)
Lopinavir/ritonavir	80/219 (37)	67/161 (42)	27/78 (35)	15/30 (50)	25/53 (47)
Duration (months)					
Stavudine	15 (9–23) *N* = 187	15 (10–22) *N* = 137	14 (9–19) *N* = 70	14 (10–21) *N* = 25	17 (12–30) *N* = 42
Zidovudine	16 (10–26) *N* = 114	16 (10–27) *N* = 88	16 (11–27) *N* = 35	16 (7–27) *N* = 22	16 (10–26) *N* = 31
Lopinavir/ritonavir	18 (10–26) *N* = 76	18.5 (10–26) *N* = 66	18 (12–30) *N* = 27	18 (7–27) *N* = 15	20 (10.5–26) *N* = 24
Any ART[b]	23 (14–33) *N* = 214	22 (14–36) *N* = 157	19 (11–30) *N* = 76	22 (15–33) *N* = 30	30 (17–41) *N* = 51
Lipids (mmol/liter)	*N* = 217	*N* = 159	*N* = 78	*N* = 30	*N* = 51
Total cholesterol	4.34 (3.75–5.25)	4.33 (3.79–5.28)	4.34 (3.86–5.15)	4.23 (3.95–4.72)	4.45 (3.66–5.49)
LDL-C	2.70 (2.20–3.22)	2.71 (2.20–3.20)	2.71 (2.20–3.08)	2.75 (2.21–3.10)	2.80 (2.19–3.49)
HDL-C	1.03 (0.82–1.30)	1.03 (0.81–1.29)	1.07 (0.88–1.27)	0.96 (0.80–1.29)	1.01 (0.75–1.32)
Triglycerides	1.11 (0.85–1.49)	1.11 (0.85–1.59)	1.07 (0.87–1.41)	0.99 (0.78–1.62)	1.23 (0.87–1.81)
Glucose (mmol/liter)					
Fasting	5.3 (4.8–5.6) *N* = 217	5.1 (4.8–5.6) *N* = 159	5.0 (4.7–5.4) *N* = 78	5.3 (4.9–5.6) *N* = 30	5.1 (4.8–5.6) *N* = 51
2 h	6.1 (5.2–7.1) *N* = 215	6.0 (5.2–7.3) *N* = 157	5.8 (5.05–6.95) *N* = 76	6.15 (5.70–7.80) *N* = 30	6.2 (5.3–7.5) *N* = 51
Lactate (mmol/liter)	1.9 (1.3–2.5) *N* = 207	1.9 (1.3–2.5) *N* = 155	1.9 (1.3–2.5) *N* = 75	1.9 (1.7–2.5) *N* = 29	1.8 (1.3–2.8) *N* = 51
Percentage limb fat (%)[c]	17 (12–21) *N* = 172	17 (12–21) *N* = 131	18 (14–21) *N* = 62	13 (9–20) *N* = 28	17 (13–21) *N* = 41
DSP *n/N* (%)	149/219 (68)	106/161 (65)	54/78 (69)	21/30 (70)	31/53 (58)

[a]Chi-square test shows that the L2 major haplogroup had fewer females than other haplogroups ($p = 0.04$).
[b]Kruskal–Wallis test showed that ART duration differed across the haplogroups ($p = 0.02$). Wilcoxon rank sum test showed that the L0 major haplogroup had significantly shorter ART duration than others ($p = 0.01$) and the L3 major haplogroup had significantly longer ART duration than the others ($p = 0.01$).
[c]Wilcoxon rank sum test showed that the L2 major haplogroup had significantly lower percentage limb fat than other groups. Other baseline characteristics did not significantly differ by major mtDNA haplogroup.
Data are reported in median (IQR) unless specified. When *N* is not specified, there was no missing data. BMI, body mass index; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; NRTI, nucleoside reverse transcriptase inhibitor; NNRTI, nonnucleoside reverse transcriptase inhibitor; ART, antiretroviral therapy; PI, protease inhibitor; DSP, distal sensory polyneuropathy.

Table 2. Univariate Analyses of Metabolic Complications and Major Mitochondrial Haplogroups

	Major haplogroups					
	L0		*L2*		*L3*	
Variable	*Beta coeff (95% CI)*	p	*Beta coeff (95% CI)*	p	*Beta coeff (95% CI)*	p
Total cholesterol	−0.01 (−0.47–0.27)	0.59	−0.12 (−0.51–0.26)	0.53	0.20 (−0.25–0.65)	0.38
Triglycerides	−0.10 (−0.34–0.13)	0.39	−0.11 (−0.35–0.13)	0.35	0.20 (−0.06–0.46)	0.13
Fasting glucose	−0.18 (−0.63–0.27)	0.42	−0.06 (−0.44–0.30)	0.73	0.26 (−0.40–0.91)	0.44
2 h glucose	−0.69 (−1.60–0.22)	0.14	0.32 (−0.62–1.27)	0.50	0.56 (−0.70–1.81)	0.38
Lactate	−0.07 (−0.38–0.24)	0.65	−0.01 (−0.35–0.33)	0.95	0.09 (−0.28–0.46)	0.64

Fasting glucose and lipid data were available from 159 participants. For 2 h glucose and lactate, data were available only from 157 and 155 participants, respectively. All variables were measured in mmol/liter. *p* values are comparing variables for each haplogroup with the other two haplogroups combined.

regimen also did not show significant associations [males: unadjusted OR 1.17 (95% CI 0.24–5.72), $p = 0.85$; females: unadjusted OR 1.28 (95% CI 0.46–3.61), $p = 0.64$].

Distal sensory polyneuropathy (DSP) and mitochondrial haplogroups

The overall prevalence of DSP was 66% (Table 1). There were no statistically significant associations between DSP and mitochondrial haplogroups, either when treated as a composite variable or when assessing symptomatic and asymptomatic DSP separately (all $p > 0.1$; data not shown).

Discussion

We explored associations between African mtDNA haplogroups and ART-related complications in a cohort of HIV-infected South African adults. We found that the L3e1 subhaplogroup was significantly associated with hypertriglyceridemia independent of lopinavir/ritonavir exposure. This is the first report to link an African mtDNA variant to hypertriglyceridemia. We found no associations between mtDNA haplogroups and other metabolic complications, including hypercholesterolemia, dysglycemia, lipoatrophy, or DSP.

Mitochondrial DNA haplogroups have been mostly studied to understand human evolution and migration.[28] However, recent studies have investigated the association of mtDNA haplogroups with human diseases and longevity.[7–15] We described the frequencies of the mitochondrial haplogroups in our South African cohort recruited from two Cape Town clinics, the majority of which belonged to the L0 haplogroup. There are limited data regarding the distribution of the mitochondrial haplogroups in the South African population. However, the distribution of mitochondrial haplogroups we found was similar to a recently published South African study conducted in 71 children.[29] None of the participants analyzed belonged to the European mitochondrial haplogroups.

The association between L3e1 subhaplogroup and hypertriglyceridemia was independent of LPV/r use, which is a well-documented cause of elevated triglycerides.[30] In a recent study of 174 non-Hispanic white HIV-infected clinical trial participants in the United States, the European mtDNA haplogroup I was associated with a decrease in triglycerides over 96 weeks of ART when compared with non-I mtDNA haplogroups.[10] To the best of our knowledge, the association between African mtDNA haplogroups and hypertriglyceridemia has not previously been studied.

Table 3. Univariate and Multivariate Analyses of Metabolic Complications and Major Haplogroups

	Major haplogroups											
	L0				*L2*				*L3*			
	Univariate		*Multivariate*		*Univariate*		*Multivariate*		*Univariate*		*Multivariate*	
Variable	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*	p	*OR (95% CI)*	p
Hypercholesterolemia	0.88 (0.45–1.74)	0.72	0.95 (0.48–1.91)	0.90	0.63 (0.25–1.59)	0.33	0.59 (0.23–1.47)	0.26	1.5 (0.76–3.14)	0.23	1.51 (0.74–3.07)	0.23
Hypertriglyceridemia	0.44 (0.19–1.04)	0.06	0.56 (0.22–1.40)	0.21	0.83 (0.29–2.40)	0.73	0.63 (0.21–1.94)	0.43	2.58 (1.14–5.83)	0.02	2.44 (1.03–5.77)	0.04
Dysglycemia	0.77 (0.40–1.49)	0.44	0.82 (0.41–1.63)	0.57	1.62 (0.72–3.66)	0.24	1.58 (0.72–3.48)	0.26	0.95 (0.47–1.92)	0.88	0.89 (0.42–1.87)	0.76
Hyperlactatemia	0.96 (0.47–1.94)	0.92	0.93 (0.45–1.96)	0.85	0.95 (0.39–2.35)	0.92	0.90 (0.35–2.30)	0.83	1.08 (0.51–2.26)	0.84	1.16 (0.54–2.49)	0.96

Adjusted for age, sex, lopinavir treatment, and treatment duration. *p* values are comparing variables for each haplogroup with the other two haplogroups combined.

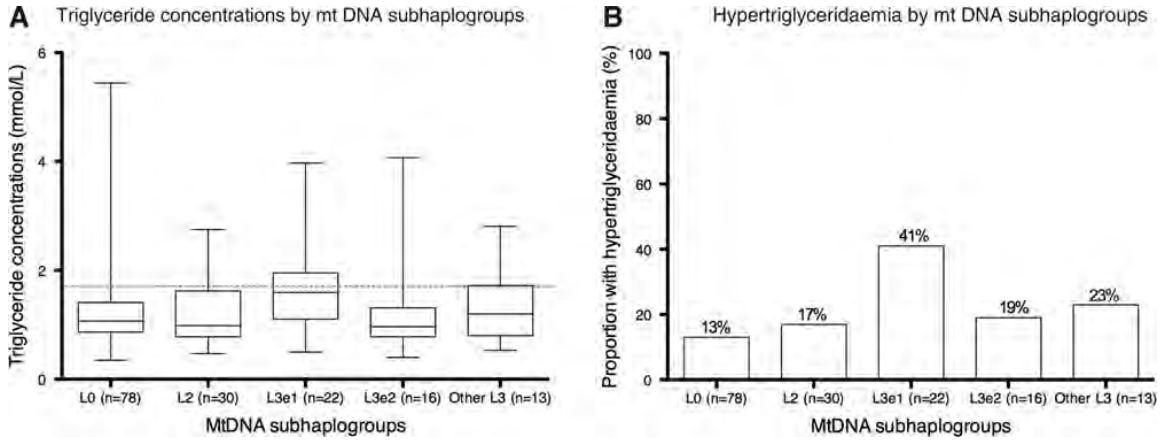

FIG. 2. Fasting triglyceride concentrations and hypertriglyceridemia by mitochondrial DNA (mtDNA) subhaplogroups. **(A)** Box plots of triglyceride concentrations in the L3e1 subhaplogroup, other L3 subhaplogroups, as well at the other haplogroups L0 and L2. Boxes represents medians and interquartile ranges; whiskers are the range. Triglycerides differed by mtDNA haplogroups (Kruskal–Wallis $p=0.047$), with L3e1 having significantly higher concentrations than other haplogroups (Wilcoxon $p=0.003$). The gray dashed line indicates the cutoff value of 1.7 mmol/liter used to categorize hypertriglyceridemia.[25] **(B)** The proportion of participants with hypertriglyceridemia by mtDNA subhaplogroups, with 41% in the L3e1 subhaplogroup and 15% in other subhaplogroups combined (Chi square test $p=0.004$).

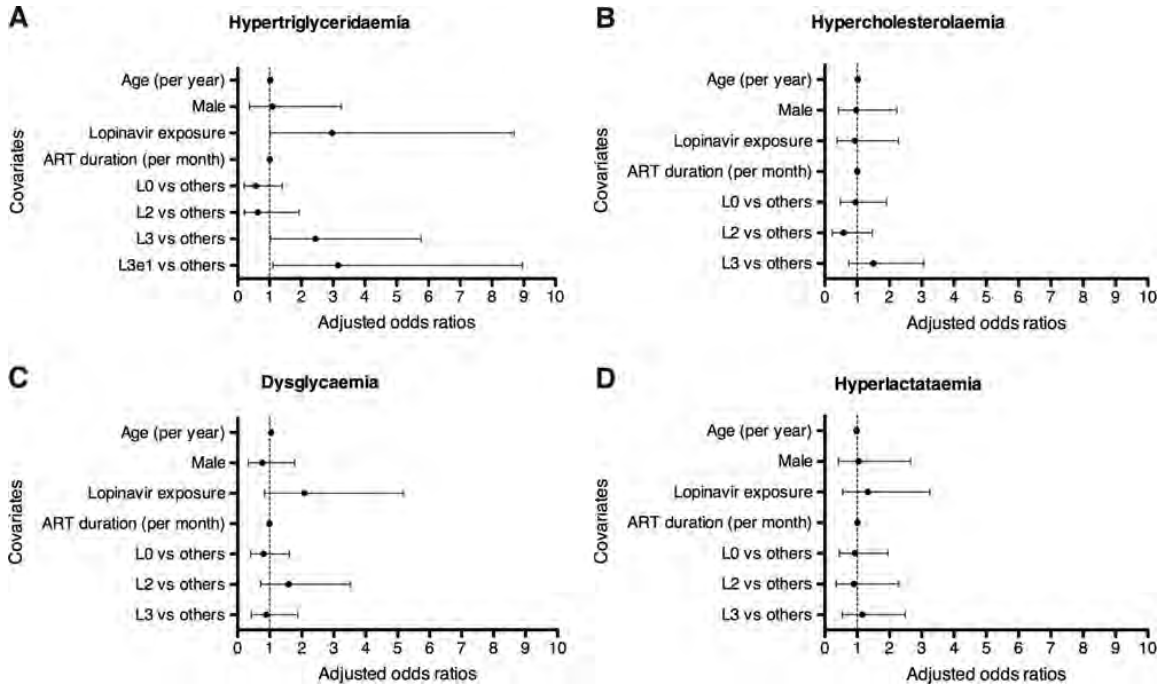

FIG. 3. Associations between mitochondrial DNA subhaplogroups and metabolic complications in HIV-infected South African adults on antiretroviral therapy (ART), shown as adjusted odds ratios (aORs). aORs were obtained from separate models: L0, L2, L3, and L3e1. The covariates age, sex, lopinavir/ritonavir (LPV/r) exposure, and ART duration displayed in **(A)** are from the L3e1 model. **(B–D)** The same covariates are from the L0 model. After adjusting for age, sex, LPV/r exposure, and ART duration, L3 [aOR 2.44 (95% CI: 1.03–5.77) and $p=0.04$], L3e1 [aOR 3.16 (95% CI: 1.11–8.96); $p=0.03$] were independently associated with hypertriglyceridemia. LPV/r exposure [aOR 2.97 (95% CI: 1.02–8.69); $p=0.047$] was also independently associated with hypertriglyceridemia and this was consistent in all subhaplogroup models.

An association between an mtDNA polymorphism and plasma triglycerides was previously reported in a Canadian cohort.[31] Variation at mtDNA position 16517 was associated with significantly higher fasting plasma triglyceride concentrations in this population. These data support the hypothesis that mitochondrial DNA variation may influence fatty acid and lipid metabolism. Fatty acids derived from plasma triglycerides are precursors of acyl CoA, which is used in the mitochondrial ß oxidation cycle of fatty acid metabolism. Genetic variation affecting mitochondrial function might also affect the utilization of fatty acid in ß oxidation.[31] Differences in cellular utilization may then alter the demand of fatty acids from triglycerides, which may influence plasma concentrations.[31] However, the exact mechanism is still unclear.

We found no association between mitochondrial DNA haplogroups and hypercholesterolemia. In the study referenced above, European mtDNA haplogroup I was associated with higher baseline (pre-ART) non-HDL cholesterol and a significant decrease in non-HDL cholesterol after 96 weeks of ART.[10] In a study of 83 elite athletes and 61 patients with sedentary lifestyles, mtDNA clade HV was significantly associated with higher LDL cholesterol in the elite athletes, but not in those with a sedentary lifestyle.[19] The lack of an association between mtDNA haplogroups and hypercholesterolemia observed in our cohort may be due to several factors. First, the studies discussed above investigated only European haplogroups,[10] not African haplogroups. Second, nuclear genetic factors known to influence cholesterol (e.g., *LPL, APOC3,* and *APOA2*) may have a stronger influence on cholesterol than mitochondrial genetic variation, or mediate relationships between mitochondrial variation and phenotype. Third, data on lifestyle factors known to influence plasma cholesterol were not available in our cohort.

We observed no statistically significant associations between African mtDNA haplogroups and fasting dysglycemia. Multivariate analyses showed that older age was an independent predictor of dysglycemia, independent of haplogroup, sex, lopinavir treatment, or duration on ART. To our knowledge, this is the first study to investigate an association between this outcome and mtDNA haplogroups in an African cohort. Several inborn mitochondrial diseases include diabetes,[32] and common mitochondrial DNA mutations have also been associated with type 2 diabetes mellitus in Asians and Europeans.[33,34] Studies investigating mitochondrial haplogroups and type 2 diabetes have shown conflicting results. Although the prevalence of dysglycemia was high (33%) in our cohort, only 8 of 159 participants had diabetes and therefore diabetes was not included as an outcome in this analysis. Hyperlactatemia is a recognized consequence of mitochondrial dysfunction associated with d-drug use.[35] We found no association between African mtDNA haplogroups and hyperlactatemia, which is similar to the finding from another South African study.[36]

There are inconsistent data regarding the influence of specific European mtDNA haplogroups on lipoatrophy.[11,12] The AIDS Clinical Trials Group (ACTG) cohort found that the mtDNA haplogroup J tended to be protective against lipoatrophy.[11] The Multicenter AIDS Cohort Study (MACS), conducted in 410 U.S. men (100% white), found that mtDNA haplogroup H was significantly associated with clinically defined lipoatrophy in the arms and legs, and that mtDNA haplogroup T tended to be protective.[12] In contrast, a recent Italian study showed that mtDNA haplogroup T tended to increase the risk of lipoatrophy when compared to mtDNA haplogroup H.[37] These studies are limited by diverse populations and heterogeneous outcome ascertainment. It is hypothesized that variations in mtDNA haplogroups may influence lipoatrophy at the adipocyte level, affecting cellular energy production efficiency, reactive oxygen species generation, and/or levels of apoptosis, all of which may be exacerbated by use of NRTIs. However, the exact mechanism remains unclear. The lack of an association between African mtDNA haplogroups and lipoatrophy could have been influenced by the way lipoatrophy was defined in our cohort. Due to significant differences between men and women, lipoatrophy was defined as percentage limb fat below the median in the sex-stratified populations. We also compared groups in the lowest and highest tertiles and quartiles and found no significant associations (data not shown). Notably, only a subset had DEXA scans, therefore we had limited power to detect differences. Larger prospective studies are needed to determine if an association between lipoatrophy and African mtDNA haplogroups exists.

Finally, we found no association between African mtDNA haplogroups and DSP. A study conducted in non-Hispanic black participants from ACTG study 384 reported an association between the African mtDNA L1c and increased susceptibility to peripheral neuropathy during NRTI treatment.[15] As discussed above, the L1 haplogroup was found in only three participants in our cohort and they were excluded from our analyses, so we were unable to replicate this reported association. Here, DSP was frequent and no association was observed with a particular haplogroup. We were also unable to adjust for the effect of d-drugs, an important cause of DSP, as >90% of participants had been exposed to stavudine and/or didanosine. All participants were examined by either of two trained clinicians, and DSP was more rigorously defined compared with many other reports. The prevalence of DSP in our cohort was high (66%), compared with that reported in the ACTG study (33%).[15]

Our study had several limitations. The design was cross-sectional, with participants of different HIV disease stages and different ART durations. We excluded ART-naive participants and participants with current opportunistic infections, known hepatic or renal disease, or with known dyslipidemia (or taking lipid-lowering therapy) or dysglycemia (or taking antidiabetic drugs). Viral load data were missing in the majority of participants, and were therefore not included in the analysis as a covariate. Our sample size was relatively small and we did not formally adjust for multiple comparisons. However, when considering the five mtDNA subhaplogroups we analyzed, only the unadjusted analyses between subhaplogroup L3e1 and triglycerides concentrations (Wilcoxon $p=0.003$), or hypertriglyceridemia ($p=0.007$) remained significant when our level of significance was corrected to $p<0.01$.

In conclusion, we observed a novel association between African mtDNA subhaplogroup L3e1 and hypertriglyceridemia. Functional studies are needed to unravel the mechanism by which this subhaplogroup may increase triglycerides. We did not find associations between African mtDNA haplogroups and the other ART-related complications studied, but we may have been underpowered to identify smaller associations. Larger studies in sub-Saharan Africa, which has

the highest HIV burden in the world but few pharmacogenomic studies, are needed to confirm the association we found between African mtDNA subhaplogroup L3e1 and hypertriglyceridemia, and to explore associations between mtDNA haplogroups and other HIV- and ART-related complications.

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References

1. Kohler JJ and Lewis W: A brief overview of mitochondrial toxicity from NRTIs. Environ Mol Mutagen 2007;48:166–172.
2. Falutz J: HIV infection, body composition changes and related metabolic complications: Contributing factors and evolving management strategies. Curr Opin Clin Nutr Metab Care 2011;14:255–260.
3. Karamchand L, Dawood H, and Chuturgoon AA: Lymphocyte mitochondrial depolarization and apoptosis in HIV-1-infected HAART patients. J Acquir Immune Defic Syndr 2008;48:381–388.
4. Viengchareun S, Caron M, Auclair M, *et al.:* Mitochondrial toxicity of indinavir, stavudine and zidovudine involves multiple cellular targets in white and brown adipocytes. Antivir Ther 2007;12:919–929.
5. Wallace DC, Brown MD, and Lott MT: Mitochondrial DNA variation in human evolution and disease. Gene 1999;238: 211–230.
6. Saxena R, de Bakker PIW, Singer K, Mootha V, Burtt N, Hirshchorn M, *et al.:* Comprehensive association testing of common mitochondrial DNA variation. Am J Hum Genet 2006;76:54–61.
7. Grady B, Samuels DC, Robbins GK, Selph B, Canter JA, Pollard RB, *et al.:* Mitochondrial genomics and CD4 T-cell count recovery after antiretroviral therapy initiation in AIDS Clinical Trials Group Study 384. J Acquir Immune Defic Syndr 2011;58:363–370.
8. Hendrickson SL, Hutcheson HB, Ruiz-Pesini E, Poole JC, Lautenberger J, Sezgin E, *et al.:* Mitochondrial DNA haplogroups influence AIDS progression. AIDS 2008;22: 2429–2439.
9. Micheloud D, Berenguer J, Guzmán-Fulgencio M, Campos Y, Garcia-Álvarez M, Catalán P, *et al.:* European mitochondrial DNA haplogroups and metabolic disorders in HIV/HCV coinfection. J Acquir Immune Defic Syndr 2011;58:371–378.
10. Hulgan T, Haubrich R, Riddler SA, Tebas P, Ritchie MD, McComsey GA, Haas DW, and Canter JA: European mitochondrial DNA haplogroups and metabolic changes during antiretroviral therapy in AIDS Clinical Trials Group Study A5142. AIDS 2011;25:37–47.
11. Hulgan T, Tebas P, Canter JA, Mulligan K, Haas DW, Dubé M, Grinspoon S, *et al.:* Hemochromatosis gene polymorphisms, mitochondrial haplogroups, and peripheral lipoatrophy during antiretroviral therapy. JID 2008;197: 858–866.
12. Hendrickson SL, Kingsley LA, Ruiz-Pesini E, Poole JC, Jacobson LP, Palella FJ, *et al.:* Mitochondrial DNA haplogroups influence lipoatrophy after highly active antiretroviral therapy. JAIDS 2009;51:111–116.
13. Hulgan T, Haas DW, Haines JL, Ritchie MD, Robbins GK, Shafer RW, *et al.:* Mitochondrial haplogroups and peripheral neuropathy during antiretroviral therapy: An adult AIDS clinical trials group study. AIDS 2005;19:1341–1349.
14. Canter JA, Haas DW, Kallianpur AR, Ritchie MD, Robbins GK, Shafer W, *et al.:* The mitochondrial pharmacogenomics of haplogroup T: MTND2*LHON4917G and antiretroviral therapy-associated peripheral neuropathy. Pharmacogenom J 2008;8:71–77.
15. Canter JA, Robbins GK, Selph D, Clifford DB, Kallianpur AR, Shafer R, *et al.* for the ACTG 384 and New Work Concept Sheet 273 Study Teams: African mitochondrial DNA subhaplogroups and peripheral neuropathy during antiretroviral therapy. JID 2010;201:1703–1707.
16. Kokaze A, Ishikawa M, Matsunaga N, Yoshida M, Sekine Y, Teruya K, *et al.:* Association of the mitochondrial DNA 5178 A/C polymorphism with serum lipid levels in the Japanese population. Hum Genet 2001;109:521–525.
17. Lal S, Madhavan M, and Heng CK: The association of mitochondrial DNA 5178 C>A polymorphism with plasma lipid levels among three ethnic groups. Ann Hum Genet 2005;69:639–644.
18. Park KS, Chan JC, Chuang LM, Suzuki S, Araki E, Nanjo K, *et al.:* A mitochondrial DNA variant at position 16189 is associated with type 2 diabetes mellitus in Asians. Diabetologia 2008;51:602–608.
19. Dahmani Y, Marcuello A, Diez-Sanchez C, Ruiz-Pesini E, Montoya J, and Lopez-Perez J: Association of human mitochondrial DNA variants with plasma LDL levels. Mitochondrion 2008;8:247–253.
20. Feder J, Ovadia O, Blech I Cohen J, WainsteinJ, Harman-Boehm I, *et al.:* Parental diabetes status reveals association of mitochondrial DNA haplogroup J1 with type 2 diabetes. BMC Genet 2009;10:doi:10.1186/1471-2350-10-60.
21. UNAIDS report 2010. http://www.unaids.org/documents/20101123_FS_SSA_em_en.pdf. August 1, 2011.
22. Dave JA, Lambert EV, Badri M, West S, Maartens G, and Levitt NS: Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycaemia and insulin sensitivity in South African HIV-infected patients. J Acquir Immune Defic Syndr 2011;57:284–289.
23. Maritz J, Benatar M, Dave J, Harrison TB, Badri M, Levitt NS, and Heckmann JM: HIV neuropathy in South Africans: Frequency, characteristics, and risk factors. Muscle Nerve 2010;41:599–606.
24. American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care 2008;31:S55–S60.

25. Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults: Executive summary of the third report of the of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 2001;285:2489–2497.
26. McComsey GA and Yau L: Asymptomatic hyperlactataemia: Predictive value, natural history and correlates. Antiviral Ther 2004;9:205–221.
27. van Oven M and Kayser M: Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat 2009;30(2):E386–E394. http://www.phylotree.org.
28. Gonder MK, Mortensen HM, Reed FA, de Sousa A, and Tishkoff SA: Whole-mtDNA genome sequence analysis of ancient African lineages. Mol Biol Evol 2007;24:757–768.
29. van der Walt EM, Smuts I, Taylor RW, Elson JL, Turnbull DM, Louw R, and van der Westhuizen FH: Characterization of mtDNA variation in a cohort of South African paediatric patients with mitochondrial disease. Eur J Hum Genet 2012;20:650–656.
30. Montes ML, Pulido F, Barros C, Condes E, Rubio R, Cepeda C, *et al.:* Lipid disorders in antiretroviral-naïve patients with lopinavir-ritonavir based HAART: Frequency, characterization and risk factors. JAC 2005;55:800–804.
31. Hegele RA, Zinman B, Hanley JG, Harris S, and Connelly PW: A common mtDNA polymorphism associated with variation in plasma triglyceride concentration. Am J Hum Genet 1997;60:1552–1555.
32. Maechler P and Wollheim CB: Mitochondrial function in normal and diabetics ß-cells. Nature 2001;414:807–812.
33. Park KS, Chan JC, Chuang L-M, Suzuki S, Araki E, Nanjo K, *et al.:* A mitochondrial DNA variant at position 16189 is associated with type 2 diabetes mellitus in Asians. Diabetologica 2008;58:602–608.
34. Poulton J, Luan J, Macaulay V, Hennings S, Mitchell J, and Wareham J: Type 2 diabetes is associated with a common mitochondrial variant: Evidence from a population-based case-control study. Hum Mol Genet 2002;11:1581–1583.
35. Hocqueloux L, Alberti C, Feugeas J-P, Lafaurie M, Lukasiewicz E, Bagnard G, *et al.:* Prevalence, risk factors and outcome of hyperlactataemia in HIV-infected patients. HIV Med 2003;4:18–23.
36. Arenas-Pinto A, Weller I, Ekong R, Grant A, Karstaedt A, Reiss P, *et al.:* Common inherited mitochondrial DNA mutations and nucleoside reverse transcriptase inhibitor-induced severe hyperlactataemia in HIV-infected adults: An exploratory study. Antivir Ther 2012;17:275–282.
37. De Luca A, Nasi M, Di Giambenedetto S, Cozzi-Lepri A, Pinti M, Marzochetti A, *et al.:* Mitochondrial DNA haplogroups and incidence of lipodystrophy in HIV infected patients on long-term antiretroviral therapy. J Acquir Immune Defic Syndr 2012;59:113–120.

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CHAPTER 5

Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa

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Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa

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WHAT IS ALREADY KNOWN ABOUT THE SUBJECT

- The polymorphisms *CYP2B6* 516G→T (rs3745274) and 983T→C (rs28399499) are strongly associated with plasma efavirenz concentrations, but do not entirely explain interindividual variability.
- A recent genome-wide association study implicated *CYP2B6* 15582C→T (rs4803419) as an independent predictor of efavirenz trough concentrations.
- Reported pharmacokinetic associations beyond *CYP2B6* are inconsistent.

WHAT THIS STUDY ADDS

- We provide evidence to show that *CYP2B6* 15582C→T is associated with plasma efavirenz concentrations in Black South Africans, in addition to *CYP2B6* 516G→T and 983T→C.
- We show that genetic associations are consistent in adults and children.
- We found no additional associations with plasma efavirenz concentrations beyond these *CYP2B6* polymorphisms.

AIMS

Genetic factors, notably *CYP2B6* 516G→T [rs3745274] and 983T→C [rs28399499], explain much of the interindividual variability in efavirenz pharmacokinetics, but data from Africa are limited. We characterized relationships between genetic polymorphisms and plasma efavirenz concentrations in HIV-infected Black South African adults and children.

METHODS

Steady-state mid-dosing interval efavirenz concentrations were measured. We genotyped 241 polymorphisms in genes potentially relevant to efavirenz metabolism and transport, including *ABCB1*, *CYP2A6*, *CYP2B6*, *CYP3A4*, *CYP3A5*, *NR1I2* and *NR1I3*.

RESULTS

Among 113 participants (59 adults and 54 children), minor allele frequencies for *CYP2B6* 516G→T, 983T→C, and 15582C→T [rs4803419] were 0.36, 0.07, and 0.09, respectively. Based on composite *CYP2B6* 15582/516/983 genotype, there were 33 extensive metabolizer, 62 intermediate metabolizer and 18 slow metabolizer genotypes. Median (IQR) mid-dose efavirenz concentrations were 1.44 (1.21–1.93) $\mu g\ ml^{-1}$, 2.08 (1.68–2.94) $\mu g\ ml^{-1}$ and 7.26 (4.82–8.34) $\mu g\ ml^{-1}$ for extensive, intermediate and slow metabolizers, respectively. In univariate analyses, a model that included composite genotype best predicted efavirenz concentrations ($\beta = 0.28$, 95% CI 0.21, 0.35, $P = 2.4 \times 10^{-11}$). Among individual *CYP2B6* polymorphisms, 516G→T best predicted efavirenz concentrations ($\beta = 0.22$, 95% CI 0.13, 0.30, $P = 1.27 \times 10^{-6}$). There was also associations with 983T→C ($\beta = 0.27$, 95% CI 0.10, 0.44, $P = 0.002$) and 15582C→T ($\beta = 0.11$, 95% CI 0.01, 0.22, $P = 0.04$). Associations were consistent in adults and children. No other polymorphisms were independently associated with efavirenz concentrations.

CONCLUSIONS

Composite *CYP2B6* genotype based on *CYP2B6* 516G→T, 983T→C, and 15582C→T best described efavirenz exposure in HIV-infected Black South African adults and children.

Introduction

Efavirenz is extensively prescribed for HIV-1 infection worldwide. It is metabolized primarily by cytochrome P450 (CYP) 2B6 [1]. Analyses with AIDS Clinical Trials Group (ACTG) protocol 5097s first showed that the non-synonymous polymorphism *CYP2B6* 516G→T (rs3745274) was strongly associated with increased plasma efavirenz exposure [2]. Many studies have since replicated this association [3–17]. The *CYP2B6* 516G→T polymorphism is more frequent with African ancestry than with European ancestry [18], which largely explains the greater mean plasma efavirenz concentrations reported among populations of African descent [19, 20]. A less frequent *CYP2B6* polymorphism, 983T→C (rs28399499), also predicts increased plasma efavirenz exposure [9, 13, 16, 21–25]. The *CYP2B6* 983 C allele is found almost exclusively with African ancestry, where it is still much less frequent than 516G→T [18]. A recent genome-wide association study of White, Black and Hispanic adults in the United States showed that a third polymorphism, *CYP2B6* 15582C→T (rs4803419), was also associated with estimated efavirenz trough concentrations independent of 516G→T and 983T→C [26].

Additional *CYP2B6* polymorphisms suggested to affect *CYP2B6* activity have been extremely infrequent [21, 27, 28], or have not predicted plasma efavirenz exposure [2, 29, 30]. Polymorphisms in genes beyond *CYP2B6* reported to affect efavirenz pharmacokinetics include *CYP2A6* [7, 30], *CYP3A5* [2], *UGT2B7* [7] and *CAR* [31], although results have been inconsistent. The genome-wide associations study noted above did not identify additional associations in or beyond *CYP2B6* [26].

Data from Africa have repeatedly replicated the association between efavirenz exposure and both *CYP2B6* 516G→T [7–17, 24, 25] and *CYP2B6* 983T→C [9, 13, 16, 21, 24, 25], but data from Africa beyond these two polymorphisms are limited. We characterized relationships between genetic polymorphisms and plasma efavirenz concentrations among HIV-infected adults and children in South Africa.

Methods

Study participants

We included adults and children who had been enrolled in two observational studies. In the adult study, adults from the public sector antiretroviral therapy (ART) programme were enrolled in a cross sectional study to evaluate associations between plasma efavirenz concentrations and metabolic complications. Eligible participants were on efavirenz-based therapy for at least 1 month, and were excluded for renal or hepatic disease, active opportunistic infections, known diabetes or dyslipidaemia, self-reported non-adherence, pregnancy, or concomitant drugs with the potential to interact with efavirenz.

In the paediatric study, African children (aged 3–15 years and weighing more than 10 kg) on efavirenz-based therapy with or without rifampicin-based antituberculosis therapy were enrolled to evaluate the effect of rifampicin-based antituberculosis therapy on efavirenz concentrations [32]. The present analyses included only data from children who were not receiving rifampicin.

All participants self-identified as Black. This study complied with the Helsinki Declaration, was approved by institutional review boards for each site, and participants or their parents gave written informed consent or assent.

Characterization of genetic polymorphisms

Human DNA was extracted from buffy coats using the Wizard® Genomic DNA purification kit (Promega, Madison, WI, USA). A total of 241 polymorphisms (70 in *ABCB1* (also called *MDR1*), 22 in *CYP2A6*, 54 in *CYP2B6*, one in *CYP2C19*, 23 in *CYP3A4*, one in *CYP3A5*, 31 in *NR1I2* (also called *PXR*) and 39 in *NR1I3* (also called *CAR*)) were successfully genotyped in the Vanderbilt DNA Resources Core using MassARRAY® iPLEX Gold (Sequenom, Inc., San Diego, California, USA). Our strategy for genotyping was as follows: For *CYP2B6*, *ABCB1* and *NR1I2* we tagged each entire gene using SeattleSNPs [33] using a cosmopolitan strategy across populations (Yoruba, Asian, African-American, European-American and Hispanic) with a 5% allelic frequency cut-off, a 0.80 threshold for r^2, 85% data convergence for tagging polymorphisms and 70% data convergence for clustering. For *CYP2B6* we included 5 kB in each 5′ and 3′ untranslated regions (UTR), and for *ABCB1* and *NR1I2* 20 kB in each UTR. For *CYP2B6*, additional polymorphisms of interest (but that were not extremely infrequent) were added based on a previous report [3], as were polymorphisms with at least 5% allelic frequency in 20 kB of the 5′ UTR identified using Ensembl Genome Browser [34] and upstream polymorphisms possibly associated with CYP2B6 expression [35]. We also included *ABCB1* 3435C→T (rs1045642) and 2677G/T/A (rs2032582), *CYP2C19* 681G→A (rs4244285) and *CYP3A5* 6986A→G (rs776746). (*CYP2C19* 681G→A was part of the already designed multiplex assay, but was not expected to affect efavirenz exposure). The final Sequenom assay design is available upon request. Laboratory personnel with no knowledge of clinical data performed genotyping. Ample duplicate and blank assays were included to assure validity. Polymorphisms were excluded for genotyping efficiency less than 95%.

Composite *CYP2B6* 15582/516/983 genotypes were assigned as follows: extensive metabolizer genotype (15582CC-516GG-983TT or 15582CT-516GG-983TT), intermediate metabolizer genotype (15582TT-516GG-983TT, 15582CC-516GT-983TT, 15582CC-516GG-983CT, 15582CT-516GT-983TT or 15582CT-516GG-983CT) and

slow metabolizer genotype (15582CC-516TT-983TT, 15582CC-516GT-983CT or 15582CC-516GG-983CC) [26].

Efavirenz concentrations

Plasma efavirenz concentrations were measured using liquid chromatography with tandem mass spectrometry (LC/MC/MS) as previously described [36]. Intraday and interday precision ranged from 1.2 to 4.1% and 2.5 to 5.3%, respectively. The calibration range was linear from 0.1 to 15 μg ml^{-1} and accuracy ranged from 95.2 to 104.6%. Several samples were obtained on the same day from each participant. Sampling times were not pre-specified, and time of prior dose was by self-report for adults, and was reported by caregivers for children. We excluded efavirenz data from samples obtained less than 10 h or greater than 20 h post-dose, or before 1 month of efavirenz therapy, and from children within 1 month after discontinuing rifampicin. An average of measured concentrations was used when multiple samples from the same participant were obtained. The repeated measurements of efavirenz concentrations were used in exploratory multilevel mixed effects (MLME) analyses.

Statistical analysis

The pharmacokinetic data were not normally distributed. Therefore, a logarithmic (log_{10}) transformation was done. Genetic associations with log_{10} transformed average efavirenz concentrations were analyzed by univariate linear regression. The tri-allelic *ABCB1* rs2032582 was analyzed as A *vs*. not A, G *vs*. not G and T *vs*. not T. We also performed analyses adjusting for *CYP2B6* 516G→T. All tests used a 5% two-sided significance level. Analyses were performed with PLINK version 1.07 (http://pngu.mgh.harvard.edu/~purcell/plink/). Bonferroni correction was used to account for multiple testing [37]. For polymorphisms previously associated with efavirenz pharmacokinetics, the threshold to carry forward into subsequent linear regression models was $P = 0.01$.

Haplotypic blocks were defined using the D′ confidence intervals method in Haploview [38] and haplotype phases were inferred using the standard E-M algorithm in PLINK [39]. Linkage disequilibrium (LD) plots and values were generated with Haploview (www.broad.mit.edu/mpg/haploview/).

The multilevel mixed effect regression model performed in Stata 11 [40], which accounted for within individual correlations, evaluated associations of efavirenz concentrations with the three polymorphisms adjusted for effects of time post-dose and age group.

Results

Study participant characteristics

Pharmacokinetic data were available from 152 participants, of whom 113 with sufficient DNA for successful genotyping are included in the present analyses. Among 59 adults, median age was 38 years (range 20 to 59 years), median weight was 65.9 kg (range 45.2 to 104.5 kg) and 45 (76%) were female. Of participants with available plasma HIV-1 RNA data, values were <400 copies ml^{-1} in 78% of adults and in 87% of children. Characteristics of study participants are presented in Table 1.

Genetic polymorphisms

Among the 113 participants, 241 polymorphisms were successfully genotyped, of which 18 were monomorphic (i.e. no minor alleles). Each of the remaining 223 polymorphisms was in Hardy–Weinberg equilibrium based on a Bonferroni adjusted *P* value threshold of 0.0002; six had unadjusted *P* values <0.05 (*NR1I3* rs4489574, $P = 0.002$, *NR1I2* rs2461817, $P = 0.021$, *ABCB1* rs17149792, $P = 0.024$, *NR1I3* rs2502815, $P = 0.026$, *ABCB1* rs10264990, $P = 0.035$ and *CYP3A4* rs28539499, $P = 0.036$). Minor allele frequencies for the 241 polymorphisms are in Supplemental Material Table S1.

Genetic associations with efavirenz concentrations

The median plasma mid-dose efavirenz concentration was 2.03 μg ml^{-1} (interquartile range 1.46–3.46 μg ml^{-1}). In univariate linear regression models, composite *CYP2B6* 15582/516/983 genotype was most strongly associated with efavirenz concentrations ($\beta = 0.28$, 95% CI 0.21, 0.35, $P = 2.4 \times 10^{-11}$). Of the 223 polymorphisms, 15 (all in *CYP2B6*) were associated with efavirenz concentrations at $P < 0.01$. These include *CYP2B6* 516G→T ($\beta = 0.22$, 95% CI 0.13, 0.30, $P = 1.3 \times 10^{-6}$) and eight polymorphisms in LD with 516G→T at $r^2 > 0.6$. The minor allele frequency of *CYP2B6* 516G→T was 0.36, with 43 (38.1%) homozygous for GG, 58 (51.3%) heterozygous for GT and 12 (10.6%) homozygous for TT. The minor allele frequency of *CYP2B6* 983T→C was 0.07, with 98 (86.7%) homozygous for TT,

Table 1

Demographic and clinical characteristics of 113 study participants in South Africa.

	Adults	*n*	Children	*n*
Efavirenz concentration (μg ml^{-1})*	2.63 (0.67–29.53)	59	1.90 (0.30–8.47)	54
Time after dose (h)*	12.3 (11.1–14.5)	59	16.2 (14.0–17.8)	54
Drug dose (mg day^{-1})*	600 (600–600)	59	300 (200–600)	54
Age (years)*	38.0 (20.0–59.0)	59	8.2 (3.0–15.1)	54
Weight (kg)*	65.9 (45.2–104.5)	59	22.3 (13.3–46.0)	54
Female gender†	45 (76.3)	59	25 (46.3)	54
CD4 T cell count (cells mm^{-3})*	290 (74–904)	59	533 (264–1522)	16
HIV-1 RNA ≤400 copies ml^{-1}†	14 (77.8)	18	13 (86.7)	15

*Medians are shown (ranges in parentheses). †Numbers of patients are shown (percentages in parentheses).

15 (13.3%) heterozygous for TC and none homozygous for CC. The minor allele frequency of *CYP2B6* 15582C→T was 0.09, with 94 (83.2%) homozygous for CC, 18 (15.9%) heterozygous for TC and one (0.9%) homozygous for TT.

To identify independent predictors of efavirenz concentrations we performed multivariable linear regression analysis adjusted for *CYP2B6* 516G→T. By this analysis, the only additional polymorphism associated at $P < 0.01$ was *CYP2B6* 983T→C ($\beta = 0.37$, 95% CI 0.23, 0.52, $P = 2.78 \times 10^{-6}$). In an analysis that adjusted for both *CYP2B6* 516G→T and 983T→C, no additional polymorphism was associated with efavirenz concentrations at $P < 0.01$, the lowest P value being for *CYP2B6* rs28723610 ($\beta = 0.21$, 95% CI 0.04, 0.38, $P = 0.013$). There was no apparent association with *CYP2B6* 15582C→T ($\beta = 0.06$, 95% CI −0.06, 0.19, $P = 0.34$). Final multivariable linear regression models are presented in Table 2.

In the above analyses, two individuals had extreme outlier efavirenz values. One individual, a 41-year-old woman with a body mass index of 22 kg m^{-2}, had three concentrations of 28.1, 28.2 and 32.3 μg ml^{-1} (mean 29.30 μg ml^{-1}). She was homozygous for 15582CC, 516GG and 983TT. One individual, a 7-year-old boy weighing 21 kg at a height of 114 cm, had three concentrations of 0.32, 0.29 and 0.28 μg ml^{-1} (mean 0.30 μg ml^{-1}). He was heterozygous for 15582CT and homozygous for 516GG and 983TT. To characterize associations with *CYP2B6* 15582C→T further, we performed *post hoc* sensitivity analyses after censoring the two outliers. In such analyses, adjusting for both 516G→T and 983T→C, five more *CYP2B6* polymorphisms in weak LD with *CYP2B6* 516G→T showed trends toward association with low efavirenz concentrations in unadjusted analyses (Table 3), and *CYP2B6* 15582C→T was now associated with efavirenz concentrations ($\beta = 0.11$, 95% CI 0.01, 0.22, $P = 0.04$) (Table 3). Relationships between genotypes and efavirenz concentrations in all individuals are presented in Figure 1A.

In the final multivariable model, each 516 T allele was associated with a 31% increase in $\log_{10}$ efavirenz concentrations ($\beta = 0.27$, $P < 0.0001$), each 983 C allele with a 46% increase ($\beta = 0.38$, $P < 0.0001$), and each 15582 T allele with a 6% increase ($\beta = 0.06$, $P = 0.340$). These three polymorphisms explained 34% of variance in $\log_{10}$ efavirenz concentrations. A model that included only *CYP2B6* 516G→T and 983T→C also explained 34% of variance in $\log_{10}$ efavirenz concentrations. Univariate linear regression models for each individual polymorphism explained 19%, 8% and only 0.59% of variance for *CYP2B6* 516G→T, 983T→C and 15582C→T, respectively.

In the *post hoc* sensitivity analysis that censored the two outlier participants, each 516 T allele was associated with a 33% increase in $\log_{10}$ efavirenz concentrations ($\beta = 0.29$, $P < 0.0001$), each 983 C allele with a 48% increase ($\beta = 0.39$, $P < 0.0001$) and each 15582 T allele with a 12% increase ($\beta = 0.12$, $P = 0.04$), which together explained 45% of variance. A model that included only *CYP2B6* 516G→T and 983T→C explained 43% of variance. Univariate linear regression models for each individual polymorphism explained 24%, 10% and only 0.02% of variance for *CYP2B6* 516G→T, 983T→C and 15582C→T, respectively.

We also performed separate analyses among children and adults. Linear regression results for children and

Table 2

Genetic associations with efavirenz concentrations in all 113 participants.

		Unadjusted analysis		516G→T adjusted		516G→T and 983T→C adjusted	
Chromosome	Polymorphism	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
19	*CYP2B6* 516G→T	0.22 (0.13, 0.30)	**1.27×10^{-6}**	NA	NA	NA	NA
19	rs8192719	0.22 (0.13, 0.30)	1.27×10^{-6}	NA	NA	NA	NA
19	rs2279343	0.22 (0.13, 0.30)	1.79×10^{-6}	NA	NA	NA	NA
19	rs10853744	0.21 (0.13, 0.29)	2.33×10^{-6}	−0.13 (−0.70, 0.45)	0.66	−0.09 (−0.61, 0.43)	0.74
19	rs11083595	0.18 (0.10, 0.26)	2.30×10^{-5}	0.002 (−0.17, 0.17)	0.99	0.07 (−0.08, 0.23)	0.37
19	rs2054675	0.18 (0.10, 0.26)	2.30×10^{-5}	0.002 (−0.17, 0.17)	0.99	0.07 (−0.08, 0.23)	0.37
19	rs3786547	0.18 (0.10, 0.27)	2.39×10^{-5}	0.0001 (−0.17, 0.17)	1.00	0.07 (−0.09, 0.23)	0.38
19	rs892216	0.18 (0.09, 0.26)	6.71×10^{-5}	−0.006 (−0.16, 0.14)	0.93	0.05 (−0.09, 0.19)	0.49
19	rs7250873	0.16 (0.09, 0.25)	1.07×10^{-4}	−0.004 (−0.21, 0.11)	0.58	0.02 (−0.12, 0.17)	0.74
19	*CYP2B6* 983T→C	0.27 (0.10, 0.44)	1.92×10^{-3}	0.37 (0.22, 0.52)	**2.78×10^{-6}**	NA	NA
19	rs1987236	−0.16 (−0.26, −0.05)	3.12×10^{-3}	−0.06 (−0.17, 0.04)	0.25	−0.02 (−0.12, 0.08)	0.68
19	rs4803417	−0.16 (−0.27, −0.05)	5.14×10^{-3}	−0.06 (−0.18, 0.05)	0.27	−0.03 (−0.13, 0.07)	0.58
19	rs2279345	−0.14 (−0.24, −0.03)	9.37×10^{-3}	−0.04 (−0.15, 0.06)	0.39	0.004 (−0.09, 0.10)	0.93
19	rs6508966	−0.14 (−0.24, −0.03)	9.37×10^{-3}	−0.04 (−0.15, 0.06)	0.39	0.004 (−0.09, 0.10)	0.93
19	rs6508965	−0.14 (−0.27, −0.05)	9.70×10^{-3}	−0.04 (−0.14, 0.06)	0.43	0.01 (−0.08, 0.11)	0.82
19	*CYP2B6* 15582C→T*	−0.06 (−0.21, 0.08)	0.42	0.03 (−0.11, 0.17)	0.67	0.06 (−0.06, 0.19)	0.34

*SNP of interest but did not meet criteria of *P* value < 0.01.

Table 3

Genetic associations with $\log_{10}$-transformed efavirenz concentrations in all 111 participants, and excluding two outliers.

		Unadjusted analysis		516G→T adjusted		516G→T and 983T→C adjusted	
Chromosome	Polymorphism	β (95% CI)	*P* value	β (95% CI)	*P* value	β (95% CI)	*P* value
19	*CYP2B6* 516G→T	0.23 (0.15, 0.30)	**3.83×10^{-8}**	NA	NA	NA	NA
19	rs8192719	0.23 (0.15, 0.30)	3.83×10^{-8}	NA	NA	NA	NA
19	rs2279343	0.22 (0.15, 0.30)	4.33×10^{-8}	NA	NA	NA	NA
19	rs10853744	0.22 (0.14, 0.29)	8.64×10^{-8}	–0.13 (–0.64, 0.38)	0.63	–0.08 (–0.53, 0.36)	0.71
19	rs11083595	0.17 (0.09, 0.24)	2.84×10^{-5}	–0.11 (–0.26, 0.04)	0.17	–0.04 (–0.18, 0.10)	0.57
19	rs2054675	0.17 (0.09, 0.24)	2.84×10^{-5}	–0.11 (–0.26, 0.04)	0.17	–0.04 (–0.18, 0.10)	0.57
19	rs3786547	0.17 (0.09, 0.24)	2.94×10^{-5}	–0.11 (–0.27, 0.04)	0.16	–0.04 (–0.18, 0.10)	0.56
19	rs892216	0.16 (0.08, 0.24)	9.04×10^{-5}	–0.09 (–0.22, 0.05)	0.20	–0.03 (–0.15, 0.09)	0.58
19	rs7250873	0.15 (0.08, 0.23)	1.47×10^{-4}	–0.14 (–0.29, 0.0008)	0.05	–0.08 (–0.20, 0.05)	0.25
19	*CYP2B6* 983T→C	0.28 (0.12, 0.43)	6.10×10^{-4}	0.38 (0.26, 0.51)	**3.13×10^{-8}**	NA	NA
19	rs6508950	–0.14 (–0.23, –0.06)	1.28×10^{-3}	–0.10 (–0.17, –0.02)	0.02	–0.05 (–0.12, 0.02)	0.14
19	rs1987236	–0.15 (–0.25, –0.06)	1.35×10^{-3}	–0.05 (–0.14, 0.04)	0.28	–0.004 (–0.09, 0.08)	0.93
19	rs4803417	–0.16 (–0.26, –0.06)	2.43×10^{-3}	–0.05 (–0.15, 0.05)	0.30	–0.01 (–0.10, 0.08)	076
19	rs10422346	–0.16 (–0.26, –0.05)	3.58×10^{-3}	–0.06 (–0.16, 0.04)	0.27	–0.03 (–0.13, 0.07)	0.58
19	rs2279345	–0.13 (–0.23, –0.04)	4.98×10^{-3}	–0.03 (–0.13, 0.06)	0.46	0.02 (–0.06, 0.10)	0.60
19	rs6508966	–0.13 (–0.23, –0.04)	4.98×10^{-3}	–0.03 (–0.13, 0.06)	0.46	0.02 (–0.06, 0.10)	0.60
19	rs6508965	–0.13 (–0.23, –0.04)	5.20×10^{-3}	–0.03 (–0.12, 0.06)	0.53	0.03 (–0.05, 0.11)	0.47
19	rs7259758	–0.14 (–0.25, –0.04)	5.47×10^{-3}	–0.06 (–0.16, 0.04)	0.25	–0.02 (–0.11, 0.06)	0.64
19	rs1962261	–0.15 (–0.26, –0.04)	6.27×10^{-3}	–0.06 (–0.16, 0.04)	0.23	–0.02 (–0.12, 0.02)	0.60
19	rs11671243	–0.12 (–0.22, –0.03)	9.16×10^{-3}	–0.02 (–0.11, 0.07)	0.68	0.02 (–0.06, 0.10)	0.55
19	*CYP2B6* 15582C→T*	–0.01 (–0.15, 0.13)	0.87	0.08 (–0.04, 0.21)	0.18	0.12 (0.01, 0.22)	**0.04**

*SNP of interest, did not meet criteria of *P* value < 0.01.

adults are shown in Supplemental Material Table S2 and S3, respectively. In both analyses, *CYP2B6* 516G→T (and polymorphisms in strong LD with *CYP2B6* 516G→T) and 983T→C were strongly associated with $\log_{10}$ efavirenz concentrations. In unadjusted analyses, there was no association with *CYP2B6* 15582C→T. In adults, unadjusted analyses revealed that *NR1I2* rs9847782 had the greatest effect size (β = 0.37, 95% CI 0.11, 0.62, $P = 7.3 \times 10^{-3}$), which was no longer apparent when one outlier was excluded (a 41-year-old with very high efavirenz concentrations and homozygous for *NR1I2* rs9847782TT). Interestingly, in children only, when the outlier with an exceptionally low $\log_{10}$ efavirenz concentration was excluded (a 7-year-old heterozygous for *CYP2B6* 15582 CT), *CYP2B6* 15582C→T showed a trend towards significance when adjusted for *CYP2B6* 516G→T and 983T→C (β = 0.08, 95% CI 0.005, 0.30, $P = 0.05$). Relationships between genotypes and efavirenz concentrations in children and adults are presented in Figure 1B and Figure 1C, respectively.

As there were clinical differences (including age and sampling time post-dose) between adults and children in our analyses, we explored multilevel mixed effects modelling using each measured efavirenz value separately in each participant, rather than the mean of concentration values. We fitted a hierarchical model that predicted $\log_{10}$ efavirenz concentrations as a function of 1) fixed effects of age group, time after dose, *CYP2B6* 516G→T, 983T→C and 15582C→T and 2) random effects for the individual to account for within individual correlations. In this model, compared with individuals in the lowest concentration stratum (i.e. 15582CC-516GG-983TT, β = 0.58), when time after dose and age group were held constant, homozygosity for *CYP2B6* 516TT was associated with an 0.64 increase in $\log_{10}$ efavirenz concentrations (2.9-fold increase in measured efavirenz concentrations), heterozygosity for *CYP2B6* 983TC with an 0.36 increase in $\log_{10}$ efavirenz concentrations (2.1-fold increase in measured efavirenz concentrations), heterozygosity for *CYP2B6* 516GT with an 0.19 increase in $\log_{10}$ efavirenz concentrations (1.5-fold increase in measured concentrations), homozygosity for *CYP2B6* 15582TT with an 0.21 increase in $\log_{10}$ efavirenz concentrations (1.4-fold increase in measured efavirenz concentrations) and heterozygosity for *CYP2B6* 15582CT with an 0.06 increase in $\log_{10}$ efavirenz concentrations (1.2-fold increase in measured efavirenz concentrations).

Linkage disequilibrium between CYP2B6 polymorphisms

To understand relationships between *CYP2B6* polymorphisms associated with efavirenz concentrations better, we considered LD. In the first model, without adjusting

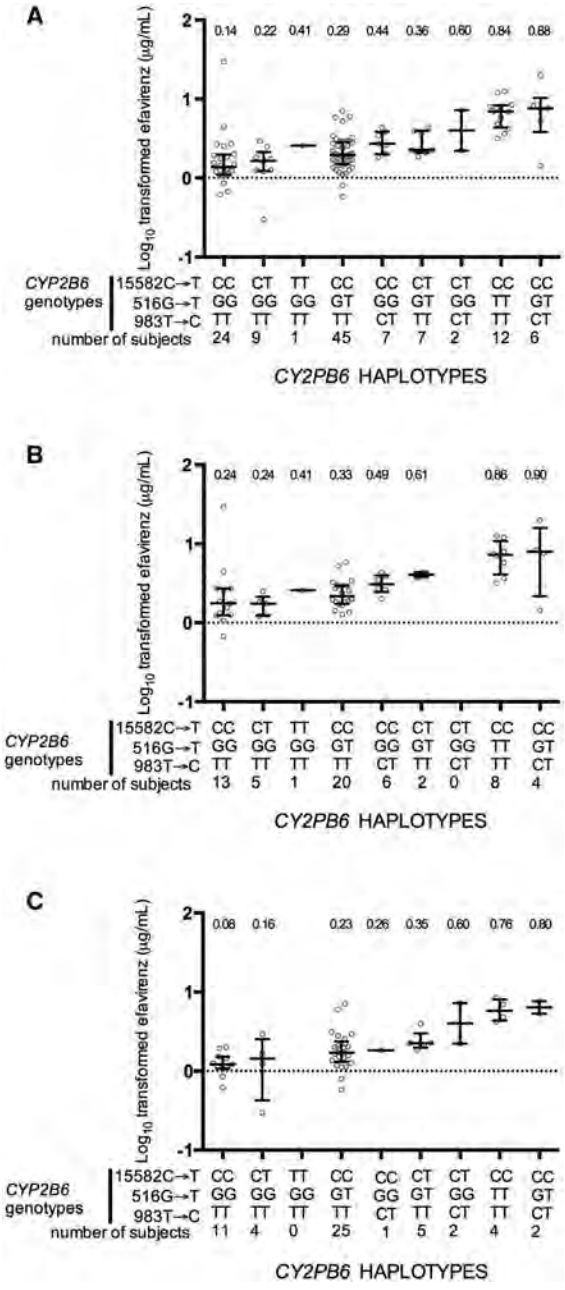

Figure 1

Relationships between *CYP2B6* polymorphisms and $\log_{10}$ transformed efavirenz concentrations in adults and children. Efavirenz concentrations were determined as described in Methods. Relationships with *CYP2B6* polymorphisms and $\log_{10}$ efavirenz concentrations are shown in all participants (A), adults (B) and children (C). On the x-axis, *CYP2B6* haplotypes represent (in order) *CYP2B6* 15582C→T (CC, CT, TT), 516G→T (GG, GT, TT) and 983T→C (TT, TC). Number per *CYP2B6* haplotype is also displayed. On the y-axis, the $\log_{10}$ transformed efavirenz concentrations are displayed. On each graph, each marker represents a different participant. Horizontal bars are medians and interquartile ranges. Median values are above

for any polymorphisms, two groups of polymorphisms were associated with efavirenz concentrations (Table 2), the first comprising eight polymorphisms in strong LD with *CYP2B6* 516G→T and with minor alleles associated with higher efavirenz concentrations (i.e. positive β values), and the second comprising five polymorphisms in weaker LD with *CYP2B6* 516G→T and with minor alleles trending towards an association with lower efavirenz concentrations (i.e. negative β values). In the second analyses, which adjusted for *CYP2B6* 516G→T, none of the above 13 polymorphisms remained associated with efavirenz concentrations, with only *CYP2B6* 983T→C becoming significant, with a greater effect size and even lower *P* value (Table 2). No polymorphisms were in strong LD with *CYP2B6* 983T→C. In the third analyses, which adjusted for *CYP2B6* 516G→T and 983T→C, no other polymorphisms were associated with efavirenz concentrations (Table 2). Linkage disequilibrium between the *CYP2B6* polymorphisms is shown in Figure 2. We only show polymorphisms with *P* values < 0.01 on unadjusted analyses, as well as *CYP2B6* 15582C→T and *CYP2B6* -2320T→C (rs7251950) polymorphisms. The latter was included because it was reported to be in strong LD with *CYP2B6* 15582C→T in other populations [26]. In our cohort, it was in weak LD with *CYP2B6* 15582C→T (r^2 =0.19).

Previously reported associations beyond CYP2B6

Polymorphisms in genes beyond *CYP2B6* reported to affect efavirenz pharmacokinetics include *ABCB1* [12, 41], *CYP2A6* [7, 30], *CYP3A5* [2], *UGT2B7* [7] and *CAR* [42, 43]. Univariate and multivariate analyses revealed no associations beyond *CYP2B6* and the results are shown in Supplemental Material Table S4. The minor allele frequencies are also displayed. The *CYP2A6* rs1801272 was monomorphic in our cohort. With only 18 participants with *CYP2B6* slow metabolizer genotypes, we were unable to replicate a previously reported association with *CYP2A6* -48T→G (rs28399433) in this group (*P* = 0.89).

Discussion

Efavirenz is one of the most extensively prescribed medications worldwide for HIV-1 infection, and multiple previous studies have associated *CYP2B6* 516 G→T [2–13] and *CYP2B6* 983 T→C [9, 13, 16, 21–25], with increased plasma efavirenz concentrations. Our study replicated these associations in Black South Africans. In addition, we showed for the first time that *CYP2B6* 15582C→T was associated with plasma efavirenz concentrations in Black South Africans, which had previously only been reported for efavirenz in one study from the United States [26]. In univariate analyses, a model that included composite genotype best predicted efavirenz concentrations. These associations

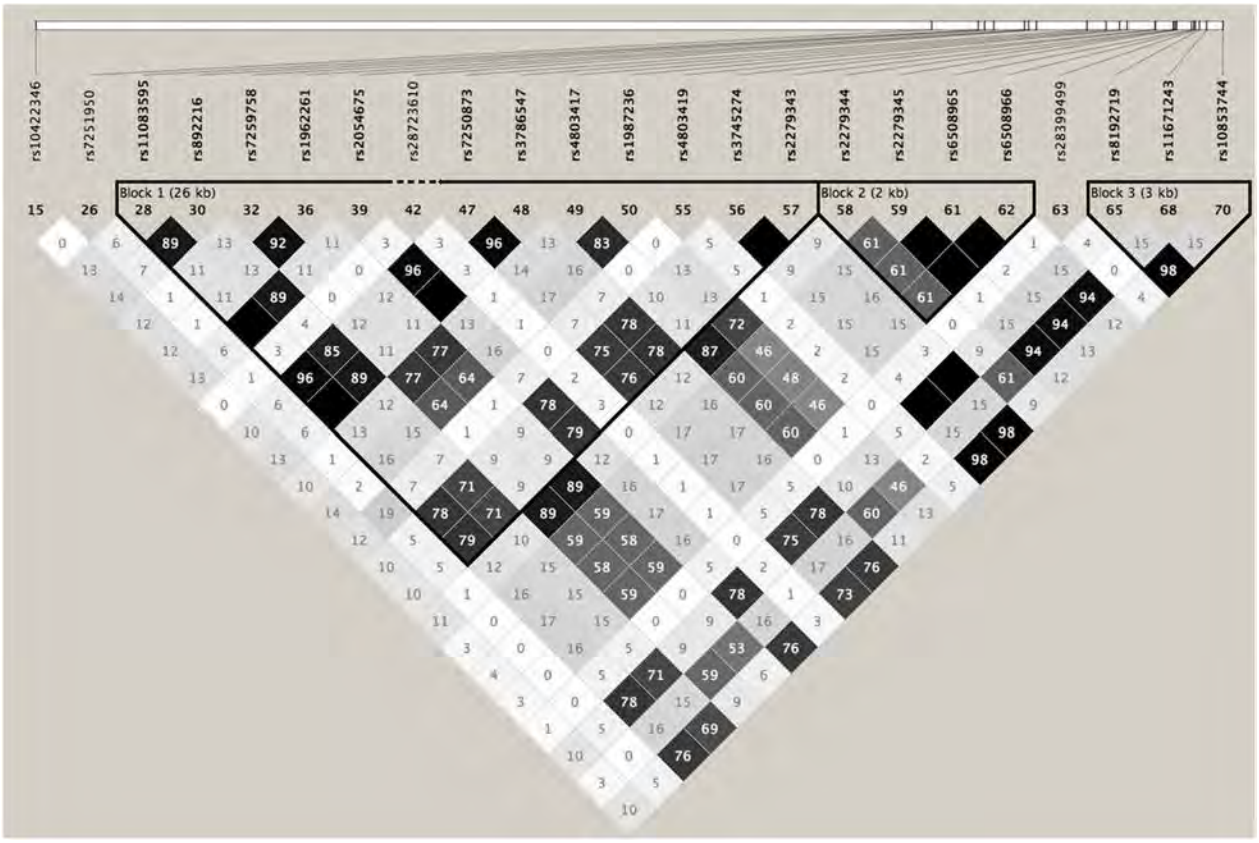

Figure 2

Linkage disequilibrium plot between polymorphisms in the *CYP2B6* locus created in Haploview. Data from all 113 participants are included. Black denotes $r^2 = 1$, shades of grey, $0 < r^2 < 1$, white, $r^2 = 0$. Only polymorphisms with *P* values <0.01 on unadjusted analyses, as well as *CYP2B6* 15582C→T and *CYP2B6* -2320T→C (rs7251950) polymorphisms are shown. r^2 is displayed to show LD

were consistent in adults and children. An association between *CYP2B6* 15582C→T and slower plasma drug clearance has also been reported among Cambodians for the CYP2B6 substrate nevirapine [44], further supporting the validity of our finding. We did not find significant associations beyond *CYP2B6* 516G→T, 983T→C and 15582C→T. Although one analysis suggested an association with *NR1I2* rs9847782, this was no longer apparent after excluding one outlier, suggesting a spurious association. Our study also provided information regarding minor allele frequencies for *ABCB1*, *CYP2A6*, *CYP2B6*, *CYP3A4*, *NR1I2* and *NR1I3* polymorphisms in a South African population.

We observed somewhat lower efavirenz concentrations in children, which is probably explained by the higher clearance in children compared with adults relative to their size [45]. Although WHO weight-based dosing in children is intended to achieve efavirenz concentrations similar to those in adults with fixed dosing, high prevalence of low efavirenz concentrations in children has been observed in a South African population similar to ours, and has been attributed to the current weight based WHO guidelines [46]. We assessed associations between polymorphisms and efavirenz concentrations separately in adults and children. In both children and adults, *CYP2B6* 516G→T had the strongest overall association with efavirenz concentrations, although *CYP2B6* 983T→C had the greatest effect size per allele. The association with *CYP2B6* 15582C→T could not be demonstrated in adults and children analyzed separately, possibly due to smaller samples sizes.

In the present analysis, minor allele frequencies of *CYP2B6* 516G→T, 983T→C, and 15882C→T were 0.36, 0.07 and 0.09, respectively. In the multivariate regression model including all three polymorphisms, *CYP2B6* 983T→C had the greatest magnitude of effect on $\log_{10}$ efavirenz concentrations ($\beta = 0.38$ for 983T→C, $\beta = 0.27$ for 516G→T, $\beta = 0.06$ for 15882C→T), but due to lower frequency only explained approximately 8% of variance in univariate analysis *vs*. 19% for 516G→T, and negligible effect for 15882C→T. In sensitivity analyses that excluded two individuals with extreme outlier efavirenz concentrations, the variance explained by all three polymorphisms increased from 34% to 45%.

Our analyses included two participants with extreme outlier efavirenz concentrations, and in neither of the cases could we identify an explanation. Dose-to-sampling times were reported to be within the 10–20 h mid-dose interval window and there were multiple efavirenz determinations from each participant. However, non-adherence in the participant with the lowest concentrations cannot be excluded. Sensitivity analyses that excluded these two participants allowed a significant association between *CYP2B6* 15882C→T and $\log_{10}$ efavirenz concentrations to be demonstrated. This same approach to censor outliers was used by Bertrand *et al*. [44] and highlights the critical importance of minimizing phenotype misclassification for detecting true associations.

Our primary analyses considered genetic associations with $\log_{10}$-transformed efavirenz concentrations. Where data from multiple samples were available from the same individual, the time adjusted average was used. We also employed the hierarchical model to consider repeated measurements taking into account variation on fixed (*CYP2B6* 516G→T, 983T→C and 15582 C→T genotypes, age group, time after dose) and random (individual) effects. The advantage of this analysis is that it provides more information about the contribution of genotype to the increase in efavirenz concentrations when all other parameters are equal.

Previous studies from Africa have replicated the association between *CYP2B6* 516G→T and plasma efavirenz exposure, including studies of patients from Zimbabwe [8, 9, 13], South Africa [10, 11, 17, 25], Ghana [7, 24], Uganda [12, 13], Tanzania [14, 15], Rwanda [16] and Ethiopia [14]. Multiple studies of patients from Africa have also shown the association with 983T→C [9, 13, 16, 21, 24, 25]. Data for association beyond *CYP2B6* are limited. A study of patients in Ghana found associations with *CYP2A6* and *UGT2B7* polymorphisms [7], but a subsequent study of patients in Ghana did not replicate independent associations with these polymorphisms [24].

The association between *CYP2B6* 15582C→T and plasma efavirenz concentrations was first discovered and replicated in a genome-wide association study from the United States [26]. This polymorphism was also associated with increased plasma efavirenz concentrations in Cambodians receiving concomitant antituberculosis therapy with isoniazid and rifampicin [47]. In Cambodians receiving nevirapine, *CYP2B6* 15582C→T was also associated with decreased plasma nevirapine clearance, as was *CYP2B6* -2320T→C (rs7251950), which was in strong LD with *CYP2B6* 15582C→T in that study [44]. In contrast, in the present study *CYP2B6* -2320T→C was in weak LD with 15582C→T ($r^2 = 0.19$) and was not associated with efavirenz concentrations. In addition, Lamba *et al*. reported that *CYP2B6* 15582C→T was associated with lower hepatic CYP2B6 expression in females [48]. In the present study, while the association with *CYP2B6* 15582C→T was only significant in analyses that included both adults and children and with outliers excluded (to provide a cleaner phenotype), this association was highly likely to be valid considering the above reports in non-African populations. Additional studies on other populations will help to replicate and refine this association further.

This study increases our understanding of efavirenz pharmacogenetics and has potential clinical implications. Because many patients (especially those with intermediate and slow metabolizer *CYP2B6* genotypes) have plasma efavirenz exposure in considerable excess of what is needed to control HIV-1 replication, there has been interest in alternative dosing strategies for efavirenz. The ENCORE1 study evaluated routinely initiating a lower dose of efavirenz (400 mg rather than 600 mg) in adults without genetic testing, and showed somewhat improved tolerability and no apparent loss of antiviral efficacy [49]. If that approach is to be translated into clinical practice, the present study suggests that the group at greatest risk for subtherapeutic efavirenz concentrations will be patients who lack polymorphisms at all three loci (i.e. *CYP2B6* 516GG-983TT-15582CC). Only by genotyping for all three polymorphism can this group be defined. Consideration has also been given to using genetic testing to individualize better efavirenz dosing. This may be most practical in paediatrics, where individualized weight-based dosing is already used. In this setting, testing for polymorphisms at all three loci will allow the most precise dosing.

The present study considered combined analyses involving both adults and children, as well as subgroup analyses involving adults and children separately. A potential concern with combined analyses is the different approach to dosing efavirenz, with adults receiving a uniform dose and children receiving a weight-based dose, which might offset the advantage of a larger sample size. Several considerations provided reassurance that combined analyses were not problematic, and improved our likelihood of finding true associations. First, associations with the known loss-of-function polymorphisms, *CYP2B6* 516G→T and 983T→C, were far more significant in combined analyses than in subgroup analyses, suggesting that our ability to detect true associations was increased, not decreased, by combining groups. Second, the association with *CYP2B6* 15582C→T only achieved significance in combined analyses. Third, the rationale for a weight-based dose in children was to approximate plasma exposure in adults, which reduces concern about combining groups.

There were limitations to the present study. Sample size limited our power to identify novel genetic associations for polymorphisms that were infrequent or had small effects, even with the dataset that included both adults and children. For example, assuming a standard deviation of 0.33 for $\log_{10}$ efavirenz concentration within each genotype group and a type II error of 0.2, we have

80% power for the following differences in pair-wise means homozygotes and heterozygotes: with 59 subjects and MAF = 0.1 a difference of 0.24 $\log_{10}$ (~75% absolute), with 59 subjects and MAF = 0.3 a difference 0.19 $\log_{10}$ (~55% absolute), with 113 subjects and MAF = 0.1 a difference of 0.17 $\log_{10}$ (~50% absolute), with 113 subjects and MAF = 0.3 a difference of 0.14 $\log_{10}$ (~40% absolute). Small sample size also limited our ability to test for interactions between *CYP2B6* genotype and age in children, although we did not anticipate such interactions. Because the dose of efavirenz was not observed, we could not be certain of dose sampling times.

In summary, the present study improves the understanding of genetic determinants of efavirenz plasma exposure in an African population, including adults and children. Studies of associations between efavirenz concentrations and polymorphisms in African populations should consider *CYP2B6* 516G→T and 983T→C, and ideally also 15582C→T.

Author contributions

PZS participated in the study design, DNA extraction, genotyping, acquisition of data, data analysis and interpretation and drafted the manuscript. PDL participated in the genotyping, data analysis and interpretation and critically revised the manuscript. HMM participated in the study design, data interpretation and critically revised the manuscript. PJS performed the analysis of the pharmacokinetic samples and helped to draft the manuscript. JAD participated in the study design, acquisition of data and critically revised the manuscript. NSL participated in study design and critically revised the manuscript. GM participated in study design, data interpretation and critically revised the manuscript. DWH participated in the study design, genotyping, data analysis and interpretation and drafted the manuscript. All authors read and approved the final manuscript.

Competing interests

All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare DWH (Principal investigator) has been a consultant to Merck. This work was supported in part by National Institute of Allergy and Infectious Diseases grants AI-077505, TR-000445 (DWH), Discovery Foundation, Welcome Trust, SA Medical Research Council and the National Health Scholar Program (PZS). The funding bodies had no role in study design, in collection, analysis and interpretation of the data, in writing of the manuscript and in the decision to submit the manuscript for publication. The rest of the authors declare no support from any organization for the submitted work and there are no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

1 Ward BA, Gorski JC, Jones DR, Hall SD, Flockhart DA, Desta Z. The cytochrome P450 2B6 (CYP2B6) is the main catalyst of efavirenz primary and secondary metabolism: implication for HIV/AIDS therapy and utility of efavirenz as a substrate marker of CYP2B6 catalytic activity. J Pharmacol Exp Ther 2003; 306: 287–300.

2 Haas DW, Ribaudo HJ, Kim RB, Tierney C, Wilkinson GR, Gulick RM, Clifford DB, Hulgan T, Marzolini C, Acosta EP. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. Aids 2004; 18: 2391–400.

3 Haas DW, Smeaton LM, Shafer RW, Robbins GK, Morse GD, Labbe L, Wilkinson GR, Clifford DB, D'Aquila RT, De Gruttola V, Pollard RB, Merigan TC, Hirsch MS, George AL Jr, Donahue JP, Kim RB. Pharmacogenetics of long-term responses to antiretroviral regimens containing efavirenz and/or nelfinavir: an Adult Aids Clinical Trials Group Study. J Infect Dis 2005; 192: 1931–42.

4 Rotger M, Colombo S, Furrer H, Bleiber G, Buclin T, Lee BL, Keiser O, Biollaz J, Decosterd L, Telenti A, Swiss HIVCS. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics 2005; 15: 1–5.

5 Almond LM, Hoggard PG, Edirisinghe D, Khoo SH, Back DJ. Intracellular and plasma pharmacokinetics of efavirenz in HIV-infected individuals. J Antimicrob Chemother 2005; 56: 738–44.

6 Rodriguez-Novoa S, Barreiro P, Rendon A, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Influence of 516G>T polymorphisms at the gene encoding the CYP450-2B6 isoenzyme on efavirenz plasma concentrations in HIV-infected subjects. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2005; 40: 1358–61.

7 Kwara A, Lartey M, Sagoe KW, Kenu E, Court MH. CYP2B6, CYP2A6 and UGT2B7 genetic polymorphisms are predictors of efavirenz mid-dose concentration in HIV-infected patients. Aids 2009; 23: 2101–6.

8 Nyakutira C, Roshammar D, Chigutsa E, Chonzi P, Ashton M, Nhachi C, Masimirembwa C. High prevalence of the CYP2B6 516G-->T(*6) variant and effect on the population pharmacokinetics of efavirenz in HIV/AIDS outpatients in Zimbabwe. Eur J Clin Pharmacol 2008; 64: 357–65.

9 Maimbo M, Kiyotani K, Mushiroda T, Masimirembwa C, Nakamura Y. CYP2B6 genotype is a strong predictor of systemic exposure to efavirenz in HIV-infected Zimbabweans. Eur J Clin Pharmacol 2012; 68: 267–71.

10 Cohen K, Grant A, Dandara C, McIlleron H, Pemba L, Fielding K, Charalombous S, Churchyard G, Smith P, Maartens G.

Effect of rifampicin-based antitubercular therapy and the cytochrome P450 2B6 516G>T polymorphism on efavirenz concentrations in adults in South Africa. Antivir Ther 2009; 14: 687–95.

11 Gounden V, van Niekerk C, Snyman T, George JA. Presence of the CYP2B6 516G> T polymorphism, increased plasma efavirenz concentrations and early neuropsychiatric side effects in South African HIV-infected patients. AIDS research and therapy 2010; 7: 32.

12 Mukonzo JK, Roshammar D, Waako P, Andersson M, Fukasawa T, Milani L, Svensson JO, Ogwal-Okeng J, Gustafsson LL, Aklillu E. A novel polymorphism in ABCB1 gene, CYP2B6*6 and sex predict single-dose efavirenz population pharmacokinetics in Ugandans. Br J Clin Pharmacol 2009; 68: 690–9.

13 Jamshidi Y, Moreton M, McKeown DA, Andrews S, Nithiyananthan T, Tinworth L, Holt DW, Sadiq ST. Tribal ethnicity and CYP2B6 genetics in Ugandan and Zimbabwean populations in the UK: implications for efavirenz dosing in HIV infection. J Antimicrob Chemother 2010; 65: 2614–9.

14 Ngaimisi E, Habtewold A, Minzi O, Makonnen E, Mugusi S, Amogne W, Yimer G, Riedel KD, Janabi M, Aderaye G, Mugusi F, Bertilsson L, Aklillu E, Burhenne J. Importance of ethnicity, CYP2B6 and ABCB1 genotype for efavirenz pharmacokinetics and treatment outcomes: a parallel-group prospective cohort study in two sub-Saharan Africa populations. PLoS One 2013; 8: e67946.

15 Ngaimisi E, Mugusi S, Minzi OM, Sasi P, Riedel KD, Suda A, Ueda N, Janabi M, Mugusi F, Haefeli WE, Burhenne J, Aklillu E. Long-term efavirenz autoinduction and its effect on plasma exposure in HIV patients. Clin Pharmacol Ther 2010; 88: 676–84.

16 Mutwa PR, Fillekes Q, Malgaz M, Tuyishimire D, Kraats R, Boer KR, Burger DM, van Schaik RH, Muganga N, Geelen SP. Mid-dosing interval efavirenz plasma concentrations in HIV-1-infected children in Rwanda: treatment efficacy, tolerability, adherence, and the influence of CYP2B6 polymorphisms. J Acquir Immune Defic Syndr 2012; 60: 400–4.

17 Viljoen M, Karlsson MO, Meyers TM, Gous H, Dandara C, Rheeders M. Influence of CYP2B6 516G>T polymorphism and interoccasion variability (IOV) on the population pharmacokinetics of efavirenz in HIV-infected South African children. Eur J Clin Pharmacol 2012; 68: 339–47.

18 dbSNP. Short Genetic Variations. Available at http://www.ncbi.nlm.nih.gov/projects/SNP/ (last accessed 8 February 2015).

19 Barrett JS, Joshi AS, Chai M, Ludden TM, Fiske WD, Pieniaszek HJ Jr. Population pharmacokinetic meta-analysis with efavirenz. Int J Clin Pharmacol Ther 2002; 40: 507–19.

20 Pfister M, Labbe L, Hammer SM, Mellors J, Bennett KK, Rosenkranz S, Sheiner LB, Adult ACTGS. Population pharmacokinetics and pharmacodynamics of efavirenz, nelfinavir, and indinavir: Adult AIDS Clinical Trial Group Study 398. Antimicrob Agents Chemother 2003; 47: 130–7.

21 Wang J, Sonnerborg A, Rane A, Josephson F, Lundgren S, Stahle L, Ingelman-Sundberg M. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. Pharmacogenet Genomics 2006; 16: 191–8.

22 Ribaudo HJ, Liu H, Schwab M, Schaeffeler E, Eichelbaum M, Motsinger-Reif AA, Ritchie MD, Zanger UM, Acosta EP, Morse GD, Gulick RM, Robbins GK, Clifford D, Haas DW. Effect of CYP2B6, ABCB1, and CYP3A5 polymorphisms on efavirenz pharmacokinetics and treatment response: an AIDS Clinical Trials Group study. J Infect Dis 2010; 202: 717–22.

23 Wyen C, Hendra H, Vogel M, Hoffmann C, Knechten H, Brockmeyer NH, Bogner JR, Rockstroh J, Esser S, Jaeger H, Harrer T, Mauss S, van Lunzen J, Skoetz N, Jetter A, Groneuer C, Fatkenheuer G, Khoo SH, Egan D, Back DJ, Owen A. German Competence Network for HA. Impact of CYP2B6 983T>C polymorphism on non-nucleoside reverse transcriptase inhibitor plasma concentrations in HIV-infected patients. J Antimicrob Chemother 2008; 61: 914–8.

24 Sarfo FS, Zhang Y, Egan D, Tetteh LA, Phillips R, Bedu-Addo G, Sarfo MA, Khoo S, Owen A, Chadwick DR. Pharmacogenetic associations with plasma efavirenz concentrations and clinical correlates in a retrospective cohort of Ghanaian HIV-infected patients. J Antimicrob Chemother 2013; 69: 491–9.

25 Swart M, Skelton M, Ren Y, Smith P, Takuva S, Dandara C. High predictive value of CYP2B6 SNPs for steady-state plasma efavirenz levels in South African HIV/AIDS patients. Pharmacogenet Genomics 2013; 23: 415–27.

26 Holzinger ER, Grady B, Ritchie MD, Ribaudo HJ, Acosta EP, Morse GD, Gulick RM, Robbins GK, Clifford DB, Daar ES, McLaren P, Haas DW. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. Pharmacogenet Genomics 2012; 22: 858–67.

27 Rotger M, Tegude H, Colombo S, Cavassini M, Furrer H, Decosterd L, Blievernicht J, Saussele T, Gunthard HF, Schwab M, Eichelbaum M, Telenti A, Zanger UM. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. Clin Pharmacol Ther 2007; 81: 557–66.

28 Rotger MCS, Cavassini M. Genetic Variability of CYP2B6 in Individuals with Extremely High Efavirenz Plasma Concentrations. 13th Conference on Retroviruses and Opportunistic Infections; Denver, CO 2006.

29 Rotger M, Saumoy M, Zhang K, Flepp M, Sahli R, Decosterd L, Telenti A, Swiss HIVCS. Partial deletion of CYP2B6 owing to unequal crossover with CYP2B7. Pharmacogenet Genomics 2007; 17: 885–90.

30 di Iulio J, Fayet A, Arab-Alameddine M, Rotger M, Lubomirov R, Cavassini M, Furrer H, Gunthard HF, Colombo S, Csajka C, Eap CB, Decosterd LA, Telenti A, Swiss HIVCS. *In vivo* analysis of efavirenz metabolism in individuals with impaired CYP2A6 function. Pharmacogenet Genomics 2009; 19: 300–9.

31 Wyen C, Hendra H, Siccardi M, Platten M, Jaeger H, Harrer T, Esser S, Bogner JR, Brockmeyer NH, Bieniek B, Rockstroh J, Hoffmann C, Stoehr A, Michalik C, Dlugay V, Jetter A,

Knechten H, Klinker H, Skaletz-Rorowski A, Fatkenheuer G, Egan D, Back DJ, Owen A, German Competence Network for HIVAC. Cytochrome P450 2B6 (CYP2B6) and constitutive androstane receptor (CAR) polymorphisms are associated with early discontinuation of efavirenz-containing regimens. J Antimicrob Chemother 2011; 66: 2092–8.

32 McIlleron HM, Schomaker M, Ren Y, Sinxadi P, Nuttall JJ, Gous H, Moultrie H, Eley B, Merry C, Smith P, Haas DW, Maartens G. Effects of rifampin-based antituberculosis therapy on plasma efavirenz concentrations in children vary by CYP2B6 genotype. Aids 2013; 27: 1933–40.

33 SeattleSNPs. Variation Discovery Resource. Available at http://pga.gs.washington.edu (last accessed 8 February 2015).

34 Ensembl Genome Browser. Available at http://useast.ensembl.org/index.html (last accessed 8 February 2015).

35 Haas DW, Wilkinson GR, Kuritzkes DR, Richman DD, Nicotera J, Mahon LF, Sutcliffe C, Siminski S, Andersen J, Coughlin K, Clayton EW, Haines J, Marshak A, Saag M, Lawrence J, Gustavson J, Anne Bennett J, Christensen R, Matula MA, Wood AJ, Adult ACTG. A multi-investigator/institutional DNA bank for AIDS-related human genetic studies: AACTG Protocol A5128. HIV Clin Trials 2003; 4: 287–300.

36 Ren Y, Nuttall JJ, Eley BS, Meyers TM, Smith PJ, Maartens G, McIlleron HM. Effect of rifampicin on efavirenz pharmacokinetics in HIV-infected children with tuberculosis. J Acquir Immune Defic Syndr 2009; 50: 439–43.

37 Miller RGJ, ed. Simultaneous statistical inference. New York: Springer-Verlag, 1981.

38 Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 2005; 21: 263–5.

39 Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007; 81: 559–75.

40 StataCorp. 2009. Stata Statistical Software: Release 11. College Station TSL.

41 Swart M, Ren Y, Smith P, Dandara C. ABCB1 4036A>G and 1236C>T Polymorphisms affect plasma efavirenz levels in South African HIV/AIDS patients. Frontiers in genetics 2012; 3: 236.

42 Cortes CP, Siccardi M, Chaikan A, Owen A, Zhang G, la Porte CJ. Correlates of efavirenz exposure in Chilean patients affected with human immunodeficiency virus reveals a novel association with a polymorphism in the constitutive androstane receptor. Ther Drug Monit 2013; 35: 78–83.

43 Swart M, Whitehorn H, Ren Y, Smith P, Ramesar RS, Dandara C. PXR and CAR single nucleotide polymorphisms influence plasma efavirenz levels in South African HIV/AIDS patients. BMC Med Genet 2012; 13: 112.

44 Bertrand J, Chou M, Richardson DM, Verstuyft C, Leger PD, Mentre F, Taburet AM, Haas DW, Group AS. Multiple genetic variants predict steady-state nevirapine clearance in HIV-infected Cambodians. Pharmacogenet Genomics 2012; 22: 868–76.

45 Holford N, Heo YA, Anderson B. A pharmacokinetic standard for babies and adults. J Pharm Sci 2013; 102: 2941–52.

46 Ren Y, Nuttall JJ, Egbers C, Eley BS, Meyers TM, Smith PJ, Maartens G, McIlleron HM. High prevalence of subtherapeutic plasma concentrations of efavirenz in children. J Acquir Immune Defic Syndr 2007; 45: 133–6.

47 Bertrand J, Verstuyft C, Chou M, Borand L, Chea P, Nay KH, Blanc FX, Mentre F, Taburet AM, Group CS. Dependence of efavirenz- and rifampicin-isoniazid-based antituberculosis treatment drug-drug interaction on CYP2B6 and NAT2 genetic polymorphisms: ANRS 12154 study in Cambodia. J Infect Dis 2014; 209: 399–408.

48 Lamba V, Lamba J, Yasuda K, Strom S, Davila J, Hancock ML, Fackenthal JD, Rogan PK, Ring B, Wrighton SA, Schuetz EG. Hepatic CYP2B6 expression: gender and ethnic differences and relationship to CYP2B6 genotype and CAR (constitutive androstane receptor) expression. J Pharmacol Exp Ther 2003; 307: 906–22.

49 Puls RL, Amin J, Losso M, Phanuphak P, Nwizu C, Orrell C, Young B, Shahar E, Wolff M, Gazzard B, Read T, Hill A, Cooper DA, Emery S, Grp ES. Efficacy of 400 mg efavirenz versus standard 600 mg dose in HIV-infected, antiretroviral-naive adults (ENCORE1): a randomised, double-blind, placebo-controlled, non-inferiority trial. Lancet 2014; 383: 1474–82.

Supporting information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1
Minor allele frequencies for 241 polymorphisms in 113 South Africans

Table S2
Genetic associations with log10-transformed efavirenz concentrations in 54 South African children

Supplemental Table S1: Minor allele frequencies for 241 polymorphisms in 113 South Africans.

Chromosome	Polymorphism	Minor allele	Major allele	Minor allele frequency
1	rs4656994	A	G	0.36
1	rs11576415	-	C	0.00
1	rs10797094	G	A	0.17
1	rs1136207	T	C	0.02
1	rs11587213	G	A	0.19
1	rs12094497	A	G	0.17
1	rs2070902	T	C	0.33
1	rs3557	G	T	0.10
1	rs11421	C	T	0.14
1	rs4489574	T	C	0.26
1	rs2502802	G	A	0.06
1	rs6413453	T	C	0.00
1	rs5085	C	G	0.15
1	rs5082	C	T	0.10
1	rs3829793	G	C	0.02
1	rs3813628	C	A	0.02
1	rs4233368	A	C	0.44
1	rs2307418	C	A	0.00
1	rs7527212	T	G	0.25
1	rs2307424	T	C	0.06
1	rs2502815	T	C	0.23
1	rs3003596	G	A	0.40
1	rs2501873	G	A	0.28
1	rs6686001	T	G	0.01
1	rs10158043	-	G	0.00
1	rs12562860	C	T	0.24
1	rs11265571	T	A	0.20
1	rs10157822	T	C	0.29
1	rs2501870	T	C	0.19
1	rs11265572	-	G	0.00
1	rs12029543	G	T	0.20
1	rs2502807	C	A	0.04
1	rs6671288	G	C	0.30
1	rs12032041	-	T	0.00
1	rs4265446	G	A	0.23
1	rs12030706	G	A	0.07
1	rs6688368	T	C	0.27
1	rs4656998	A	G	0.04
1	rs4656999	T	C	0.04

Supplemental Table S1: Minor allele frequencies for 241 polymorphisms in 113 South Africans.

Chromosome	Polymorphism	Minor allele	Major allele	Minor allele frequency
3	rs1464599	G	A	0.17
3	rs11928653	-	A	0.00
3	rs10511394	G	T	0.17
3	rs9847782	C	T	0.05
3	rs4688035	G	A	0.20
3	rs9832958	G	A	0.23
3	rs7643038	G	A	0.48
3	rs940087	G	A	0.08
3	rs4688038	C	T	0.33
3	rs1523129	T	C	0.30
3	rs3814055	T	C	0.18
3	rs1523127	T	G	0.21
3	rs3842689	C	A	0.18
3	rs3814056	0	T	0.00
3	rs2461831	A	T	0.04
3	rs2037548	G	C	0.04
3	rs1403526	G	A	0.46
3	rs6438545	T	G	0.13
3	rs13085558	C	T	0.01
3	rs4687884	G	T	0.24
3	rs4688040	T	G	0.28
3	rs6771638	T	G	0.12
3	rs2461817	G	T	0.45
3	rs12721597	G	A	0.07
3	rs2461816	A	G	0.09
3	rs2472681	T	C	0.39
3	rs2472682	C	A	0.22
3	rs6784598	C	G	0.17
3	rs11917714	T	C	0.36
3	rs6438550	G	A	0.06
3	rs1054191	A	G	0.14
7	rs17209837	C	T	0.28
7	rs1055302	A	G	0.36
7	rs3842	G	A	0.25
7	rs28401816	G	A	0.15
7	rs28401815	G	A	0.23
7	rs28401814	T	C	0.15
7	rs6978925	T	C	0.08
7	rs2235047	G	T	0.22

Supplemental Table S1: Minor allele frequencies for 241 polymorphisms in 113 South Africans.

Chromosome	Polymorphism	Minor allele	Major allele	Minor allele frequency
7	rs1045642	T	C	0.13
7	rs4437575	G	A	0.34
7	rs10808071	G	A	0.24
7	rs1002205	G	C	0.33
7	rs17149699	T	C	0.32
7	rs28401801	T	A	0.15
7	rs1922243	C	T	0.46
7	rs4148743	A	G	0.37
7	rs2373589	A	G	0.39
7	rs10280101	C	A	0.19
7	rs7787082	G	A	0.27
7	rs6959435	G	T	0.01
7	rs2032582_A	-	T	0.00
7	rs2032582_G	T	G	0.02
7	rs2032582_T	T	G	0.02
7	rs10236274	G	A	0.26
7	rs4148738	G	A	0.17
7	rs28381958	C	A	0.36
7	rs10248420	A	G	0.36
7	rs28381940	C	T	0.15
7	rs4148736	T	C	0.44
7	rs10276603	C	T	0.15
7	rs6961882	C	T	0.43
7	rs1922242	T	A	0.43
7	rs2235035	T	C	0.21
7	rs2032588	T	C	0.17
7	rs1128503	T	C	0.13
7	rs3789244	C	A	0.18
7	rs10225464	G	A	0.18
7	rs28381873	A	G	0.32
7	rs28381869	C	G	0.32
7	rs28381868	A	G	0.18
7	rs868755	A	C	0.01
7	rs2235023	A	G	0.30
7	rs28381863	G	A	0.18
7	rs28381857	C	T	0.31
7	rs955000	C	G	0.31
7	rs956825	A	G	0.17
7	rs28381850	A	C	0.20

Supplemental Table S1: Minor allele frequencies for 241 polymorphisms in 113 South Africans.

Chromosome	Polymorphism	Minor allele	Major allele	Minor allele frequency
7	rs1016793	T	C	0.01
7	rs2235020	T	A	0.16
7	rs2235015	T	G	0.33
7	rs6969155	A	G	0.19
7	rs10264990	C	T	0.24
7	rs1202179	G	A	0.41
7	rs1989831	A	T	0.40
7	rs1202174	A	G	0.41
7	rs1202172	G	T	0.41
7	rs17327442	A	T	0.26
7	rs1202186	G	A	0.26
7	rs28381820	A	C	0.44
7	rs7802773	A	G	0.38
7	rs12535512	C	T	0.01
7	rs1858923	C	T	0.06
7	rs17149792	T	C	0.30
7	rs13233308	T	C	0.01
7	rs17149824	G	T	0.46
7	rs1978095	C	T	0.03
7	rs2157926	A	T	0.46
7	rs10267099	G	A	0.12
7	rs2188531	A	G	0.46
7	rs6972098	C	T	0.49
7	rs776746	G	A	0.14
7	rs28371763	-	A	0.00
7	rs28988599	-	T	0.00
7	rs28988593	C	T	0.09
7	rs4646437	C	T	0.12
7	rs2687117	T	C	0.31
7	rs28988586	T	C	0.04
7	rs2246709	G	A	0.33
7	rs2687116	T	G	0.21
7	rs28908769	C	A	0.03
7	rs7801671	A	C	0.23
7	rs36231115	-	G	0.00
7	rs2740574	A	G	0.23
7	rs28988568	A	C	0.23
7	rs1851426	C	T	0.22
7	rs35666940	-	C	0.00

Supplemental Table S1: Minor allele frequencies for 241 polymorphisms in 113 South Africans.

Chromosome	Polymorphism	Minor allele	Major allele	Minor allele frequency
7	rs36231117	T	C	0.01
7	rs2740571	C	T	0.25
7	rs28539499	G	A	0.24
7	rs28706178	-	C	0.00
7	rs1403194	-	A	0.00
7	rs2687102	G	A	0.26
7	rs28469324	A	T	0.15
7	rs2737418	T	G	0.29
10	rs4244285	A	G	0.20
19	rs2604862	A	G	0.14
19	rs11878604	C	T	0.22
19	rs11670760	C	T	0.01
19	rs7251418	A	G	0.11
19	rs11672809	C	T	0.37
19	rs8192729	A	G	0.03
19	rs28399454	A	G	0.06
19	rs1801272	-	T	0.00
19	rs28399440	C	T	0.02
19	rs28399435	-	G	0.00
19	rs8192720	T	C	0.03
19	rs28399433	G	T	0.07
19	rs10418304	G	A	0.07
19	rs4105144	T	C	0.44
19	rs10422346	T	C	0.15
19	rs8102683	T	C	0.25
19	rs11671041	C	A	0.00
19	rs7256108	G	T	0.03
19	rs7255443	T	C	0.03
19	rs1496402	T	A	0.37
19	rs6508950	T	C	0.26
19	rs11666974	A	T	0.03
19	rs10411962	A	G	0.16
19	rs8109818	G	A	0.34
19	rs11671108	C	A	0.07
19	rs7251950	T	C	0.08
19	rs8105382	C	T	0.33
19	rs11083595	C	G	0.42
19	rs1808682	A	G	0.09
19	rs892216	T	C	0.45

Supplemental Table S1: Minor allele frequencies for 241 polymorphisms in 113 South Africans.

Chromosome	Polymorphism	Minor allele	Major allele	Minor allele frequency
19	rs10418990	G	C	0.03
19	rs7259758	T	G	0.14
19	rs10419125	T	C	0.01
19	rs8109525	G	A	0.16
19	rs7254579	C	T	0.16
19	rs1962261	A	G	0.14
19	rs3760657	G	A	0.01
19	rs12721652	T	C	0.35
19	rs2054675	C	T	0.42
19	rs4802100	G	C	0.01
19	rs4802101	T	C	0.03
19	rs28723610	A	G	0.05
19	rs34223104	C	T	0.01
19	rs28739581	A	T	0.36
19	rs2099361	G	T	0.01
19	rs8100458	C	T	0.14
19	rs7250873	G	A	0.43
19	rs3786547	C	T	0.42
19	rs4803417	C	A	0.16
19	rs1987236	G	A	0.19
19	rs35490259	C	T	0.25
19	rs35773040	-	G	0.00
19	rs33912321	A	G	0.02
19	rs1872121	A	G	0.25
19	rs4803419	T	C	0.09
19	rs3745274	T	G	0.36
19	rs2279343	G	A	0.36
19	rs2279344	G	A	0.14
19	rs2279345	T	C	0.21
19	rs12721649	A	G	0.27
19	rs6508965	T	C	0.21
19	rs6508966	G	C	0.21
19	rs28399499	C	T	0.07
19	rs35979566	-	T	0.00
19	rs8192719	T	C	0.36
19	rs36118214	A	G	0.27
19	rs33967301	C	T	0.02
19	rs11671243	A	C	0.21
19	rs7260329	A	G	0.13

Supplemental Table S1: Minor allele frequencies for 241 polymorphisms in 113 South Africans.

Chromosome	Polymorphism	Minor allele	Major allele	Minor allele frequency
19	rs10853744	T	G	0.37
19	rs3211371	-	C	0.00
19	rs7260525	G	A	0.02
19	rs7246465	T	C	0.21
19	rs34128717	G	A	0.14
19	rs34789700	T	G	0.27
19	rs1552222	A	T	0.35

Supplemental Table S2. Genetic associations with log_{10}-transformed efavirenz concentrations in 54 South African children.

		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Chromosome	**Polymorphism**	**β(95%CI)**	***P*-value**	**β(95%CI)**	***P*-value**	**β(95%CI)**	***P*-value**
19	rs2279343	0.25(0.13-0.37)	**1.61×10^{-04}**	NA	NA	NA	NA
19	*CYP2B6* 516G→T	0.24(0.12-0.37)	2.10×10^{-04}	NA	NA	NA	NA
19	rs8192719	0.24(0.12-0.37)	2.10×10^{-04}	NA	NA	NA	NA
19	rs10853744	0.23(0.11-0.34)	3.67×10^{-04}	-0.04(-0.57 to 0.48)	0.87	-0.01(-0.45 to 0.45)	0.96
19	rs892216	0.17(0.06-0.28)	4.61×10^{-03}	-0.02(-0.20 to 0.17)	0.85	0.04(-0.12 to 0.21)	0.59
19	rs3760657	-0.83(-1.38 to -0.27)	4.74×10^{-03}	-0.67(-1.17 to -0.16)	0.01	-0.59(-1.03 to -0.16)	0.01
19	rs4802100	-0.83(-1.38 to -0.27)	4.74×10^{-03}	-0.67(-1.17 to -0.16)	0.01	-0.59(-1.03 to -0.16)	0.01
19	rs34128717	-0.28(-0.47 to -0.08)	6.74×10^{-03}	-0.19(-0.37 to -0.01)	0.05	-0.11(-0.29 to 0.05)	0.17
19	rs11083595	0.16(0.04-0.27)	8.33×10^{-03}	0.11(-0.32 to 1.00)	0.31	-0.06(-0.24 to 0.12)	0.52
19	rs2054675	0.16(0.04-0.27)	8.33×10^{-03}	0.11(-0.32 to 1.00)	0.31	-0.06(-0.24 to 0.12)	0.52
19	rs3786547	0.16(0.04-0.27)	8.94×10^{-03}	-0.11(-0.32 to 1.00)	0.30	0.07(-0.09 to 0.23)	0.38
19	rs1962261	-0.26(-0.44 to -0.07)	9.86×10^{-03}	-0.17(-0.35 to 0.003)	0.05	-0.11(-0.27 to 0.05)	0.18
19	*CYP2B6* 983T→C	0.36(0.09-0.62)	9.97×10^{-03}	0.46(0.25-0.68)	**9.77×10^{-05}**	NA	NA
19	*CYP2B6* 15582C→T*	0.02(-0.18 to 0.21)	0.88	0.11(-0.07 to 0.29)	0.23	0.07(-0.08 to 0.23)	0.38

*SNP of interest but did not meet criteria of p-value<0.01

Supplemental Table S3. Genetic associations with log_{10}-transformed efavirenz concentrations in 59 South African adults.

		Unadjusted analysis		516G→T adjusted		516G→T & 983T→C adjusted	
Chromosome	**Polymorphism**	**β(95%CI)**	***P*-value**	**β(95%CI)**	***P*-value**	**β(95%CI)**	***P*-value**
19	rs11083595	0.22(0.11-0.33)	**2.41×10^{-04}**	0.16(-0.10 to 0.41)	0.23	0.24(0.004-0.49)	0.05
19	rs2054675	0.22(0.11-0.33)	2.41×10^{-04}	0.16(-0.10 to 0.41)	0.23	0.24(0.004-0.49)	0.05
19	rs3786547	0.22(0.11-0.33)	2.41×10^{-04}	0.16(-0.10 to 0.41)	0.23	0.24(0.004-0.49)	0.05
19	*CYP2B6* 516G→T	0.20(0.10-0.31)	4.49×10^{-04}	NA	NA	NA	NA
19	rs8192719	0.20(0.10-0.31)	4.49×10^{-04}	NA	NA	NA	NA
19	rs10853744	0.20(0.10-0.31)	4.49×10^{-04}	NA	NA	NA	NA
19	rs2279343	0.20(0.09-0.31)	6.57×10^{-04}	NA	NA	NA	NA
19	rs7250873	0.20(0.10-0.31)	8.09×10^{-04}	0.07(-0.17 to 0.31)	0.58	0.15(-0.08 to 0.38)	0.21
19	rs892216	0.20(0.10-0.31)	1.50×10^{-03}	0.04(-0.20 to 0.28)	0.73	0.09(-0.14 to 0.31)	0.45
3	rs9847782	0.37(0.11-0.62)	7.30×10^{-03}	0.28(0.03 to 0.52)	0.03	0.32(0.09 to 0.55)	**8.05×10^{-03}**
19	*CYP2B6* 983T→C*	0.19(0.03-0.40)	9.17×10^{-02}	0.27(0.09-0.47)	**6.21×10^{-03}**	NA	NA
19	*CYP2B6* 15582C→T*	-0.11(-0.31 to 0.10)	0.31	-0.17(-0.21 to 0.18)	0.90	0.05(-0.13 to 0.24)	0.58

*SNP of interest but did not meet criteria of p-value<0.01

Supplemental Table S4. Genetic polymorphisms beyond CYP2B6 and association with log_{10}-transformed efavirenz concentrations in 113 South Africans.

Chromosome	Gene	Polymorphism (minor allele)	MAF	Unadjusted analysis		516G→T, 983T→C & 15582C→T adjusted	
				β(95%CI)	*P*-value	β(95%CI)	*P*-value
1	*CAR*	rs2307424 (T)	0.06	-0.03(-0.22 – 0.15)	0.74	0.08(-0.07 – 0.24)	0.31
1	*CAR*	rs2502815 (T)	0.22	-0.01(-0.11 – 0.07)	0.70	-0.08(-0.08 – 0.07)	0.84
1	*CAR*	rs3003596 (G)	0.40	-0.01(-0.04 – 0.07)	0.76	-0.02(-0.09 – 0.05)	0.54
7	*ABCB1*	rs3842 (G)	0.25	0.02(-0.08 – 0.12)	0.74	-0.01(-0.10 – 0.08)	0.79
7	*ABCB1*	rs1045642 (T)	0.13	0.10(-0.02 – 0.22)	0.10	0.10(-0.004 – 0.20)	0.06
7	*ABCB1*	rs2032582 (T/G/A)	0.02	0.24(-0.08 – 0.56)	0.14	0.28(0.13 – 0.54)	0.04
7	*ABCB1*	rs1128503 (T)	0.13	-0.01(-0.14 – 0.12)	0.87	0.01(-0.09 – 0.10)	0.89
7	*CYP3A4*	rs2740574 (A)	0.23	0.05(-0.06 – 0.09)	0.40	0.02(-0.08 – 0.11)	0.73
7	*CYP3A4*	rs4646437 (C)	0.12	0.01(-0.12 – 0.12)	0.87	0.01(-0.10 – 0.12)	0.85
7	*CYP3A5*	rs776746 (G)	0.14	0.01(-0.13 – 0.12)	0.99	-0.04(-0.14 – 0.08)	0.48
10	*CYP2C19*	rs4244285 (A)	0.20	0.01(-0.10 – 0.12)	0.85	0.01(-0.10 – 0.12)	0.85
19	*CYP2A6*	rs28399433 (G)	0.07	0.17(0.001 – 0.35)	0.05	0.11(-0.03 – 0.26)	0.13
19	*CYP2A6*	rs28399454 (A)	0.06	-0.13(0.31 – 0.04)	0.15	-0.01(-0.16 – 0.14)	0.88

CHAPTER 6

Plasma efavirenz concentrations are associated with lipid and glucose concentrations

PLASMA EFAVIRENZ CONCENTRATIONS ARE ASSOCIATED WITH LIPID AND GLUCOSE CONCENTRATIONS

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LIST OF ABBREVIATIONS

95%CI	95% Confidence Interval
ADA	American Diabetes Association
ART	Antiretroviral Therapy
BMI	Body Mass Index
CYP2B6	Cytochrome P450 isoenzyme 2B6
D:A:D	Data Collection on Adverse Events of Anti-HIV Drugs
HDL	High-density lipoprotein
HIV	Human Immunodeficiency Virus
IQR	Interquartile Range
LDL	Low-density lipoprotein
NCEP III	National Cholesterol Education Program III
NNRTI	Non-nucleoside Reverse Transcriptase Inhibitor
NRTI	Nucleoside Reverse Transcriptase Inhibitor
OGTT	Oral Glucose Tolerance Test
REC REF	Research Ethics Committee Reference number
RNA	Ribonucleic acid

ABSTRACT

OBJECTIVES:

Efavirenz-based antiretroviral therapy (ART) has been associated with dyslipidaemia and dysglycaemia, risk factors for cardiovascular disease. However, the pathogenesis is not well understood. We characterized relationships between plasma efavirenz concentrations and lipid and glucose concentrations in HIV-infected South Africans.

METHODS:

Participants on efavirenz-based ART were enrolled into a cross-sectional study. Oral glucose tolerance test was performed after an overnight fast, and plasma drawn for mid-dosing interval efavirenz, fasting total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol, and triglycerides concentrations.

RESULTS:

Among 106 participants (77 women), median age was 38 years, median CD4+ T-cell count was 322 cells/μL, median duration on ART was 18 months, and median (interquartile range) efavirenz concentration was 2.23 (1.66 to 4.10) μg/mL. On multivariable analyses (adjusting for age, sex, body mass index, and ART duration) doubling of efavirenz concentrations resulted in mean changes in mmol/L (95%CI) of: total cholesterol [0.40 (0.22 to 0.59)], LDL cholesterol [0.19 (0.04 to 0.30)], HDL cholesterol [0.14 (0.07 to 0.20)], triglycerides [0.17 (0.03 to 0.33)], fasting glucose [0.18 (0.03 to 0.33)], and 2-hour glucose concentrations [0.33 (0.08 to 0.60)]. Among 57 participants with *CYP2B6* genotype data, associations between slow metabolizer genotypes and metabolic profiles were generally consistent with those for measured efavirenz concentrations.

CONCLUSIONS:

Higher plasma efavirenz concentrations are associated with higher plasma lipid and glucose concentrations. This may have implications for long-term cardiovascular complications of efavirenz-based ART, particularly among populations with high prevalence of *CYP2B6* slow metabolizer genotypes.

Keywords: efavirenz pharmacokinetics, LDL cholesterol, HDL cholesterol, triglycerides, glucose, HIV, South Africa, antiretroviral therapy

INTRODUCTION

The non-nucleoside reverse transcriptase inhibitor (NNRTI) efavirenz is extensively prescribed and is included in the World Health Organization's preferred first-line ART regimens for HIV-1 infected adults, adolescents and children at least 3 years of age.[1] Efavirenz-based ART has been associated with the development of dysglycaemia[2] and dyslipidaemia;[3-5] specifically increases in total cholesterol: HDL cholesterol ratio, LDL cholesterol, and triglycerides.[6,7] The pathogenesis of these metabolic effects are unclear, although it has been suggested that efavirenz may contribute to mitochondrial toxicity caused by concomitant thymidine analogue nucleoside reverse transcriptase inhibitors (NRTI).[8]

Data are scant regarding relationships between plasma efavirenz concentrations and plasma glucose or lipid concentrations. There is considerable interindividual variability in plasma efavirenz exposure, which is largely explained by three *CYP2B6* loss-of-function polymorphisms.[9,10] The two polymorphisms with the greatest effect, *CYP2B6* 516G→T and *CYP2B6* 983T→C, are particularly frequent with African ancestry.[11,12] The *CYP2B6* 516G→T polymorphism is also frequent in Thai and Cambodian populations.[13,14]

We investigated whether plasma efavirenz concentrations correlated with plasma lipid and/or glucose concentrations in HIV-infected South Africans. We hypothesized that higher plasma efavirenz concentrations would be associated with higher lipid and glucose concentrations.

MATERIALS AND METHODS

Study design and participants

We conducted a prospective cross-sectional study of consecutive HIV-infected African adults who presented for routine follow up visits at one community-based (Crossroads) and one hospital-based (Groote Schuur) ART clinic in Cape Town, South Africa. Participants were recruited by convenient sampling between February 2007 and September 2008. South African ART guidelines at the time of this study recommended an NNRTI plus two NRTIs (stavudine or zidovudine, each with lamivudine) as first-line therapy. Eligible participants were on efavirenz-based ART for at least six months. Exclusion criteria included pregnancy, renal or hepatic disease, active opportunistic infections, treatment for diabetes or dyslipidaemia, and self-reported non-adherence. The study was conducted in accordance with the Declaration of Helsinki and the South African Good Clinical Practice. The University of Cape Town Research Ethics Committee approved the study (REC REF 128/2007). All participants gave written informed consent.

Clinical and laboratory evaluations

Participants were instructed to fast overnight and to document the time of the evening dose of efavirenz on the day preceding the study visit. On the study day, participants underwent an oral glucose tolerance test (OGTT). Blood was drawn at 0 and 120 minutes after ingesting 75 g of glucose in 250 mL of water, and kept on ice until centrifuged within 4 hours. Plasma for efavirenz quantification was collected into 4mL lithium heparin tubes, kept on ice until centrifuged within 4 hours, and was aliquotted and promptly frozen at −20°C, then stored at -70°C until analysis at the end of recruitment in 2008. Plasma efavirenz, fasting glucose, cholesterol, and triglyceride were quantified using the 0 minute OGTT samples.

Efavirenz was quantified by a validated method using liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Applied Biosystems MDS Sciex API 4000 tandem mass spectrometer at our ISO17025 compliant and accredited analytical laboratory as previously described.[15] The assay range of quantification was 0.05 to 20 µg/mL. Accuracy ranged from 94 to 103%. Serum glucose and lipid concentrations were determined by standard methods using the ACE Alera Clinical Chemistry System (Alfa Wassermann Diagnostic Technologies, Woerden, Netherlands). Diabetes, impaired fasting glucose, and impaired glucose tolerance were defined according to American Diabetes Association criteria.[16] Hypercholesterolaemia, high LDL cholesterol, low or high HDL, and hypertriglyceridaemia were defined according to the NCEP III criteria.[17]

Medical records were reviewed to determine duration on ART, plasma HIV-1 RNA concentrations, and CD4+ T-cell counts. Baseline CD4+ T-cell counts were recorded, and current CD4+ T-cell count was defined as the most recent value within 3 months of enrolment. Adherence was assessed using a validated 4-day adherence questionnaire administered by trained staff.[18]

Pharmacokinetic and statistical analysis

Medians (interquartile ranges) and proportions or ratios were used to describe continuous and categorical data, respectively. Scatter plots to visualize relationships between plasma efavirenz concentrations and metabolic parameters were generated using Prism version 6 (GraphPad Software, Inc., La Jolla, California, USA). Plasma efavirenz concentrations were $\log_{10}$ transformed to approximate normality. Associations between $\log_{10}$ transformed plasma efavirenz concentrations and cholesterol, triglycerides, and glucose were determined using univariate and multivariate linear regression analyses on Stata (version 11, StataCorp, College Station, Texas, USA). Univariate linear regression analyses characterized associations between $\log_{10}$ transformed association as a independent variable, and several dependent variables: fasting total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides, glucose, and 2-hour OGTT glucose. Multivariate linear regression analyses adjusted for age, sex, body mass index (BMI) and total duration on ART. These potential confounders were chosen a priori. Sensitivity analyses included adjusting for current stavudine use. Case sensitivity analyses with the participant with the highest plasma concentrations were done. Missing data were not imputed.

In a subset of participants with available *CYP2B6* genotype data, we explored relationships between *CYP2B6* polymorphisms known to predict increased plasma efavirenz concentrations, *CYP2B6* 516G>T (rs3745274), 983T>C (rs28399499) and 15582C>T (rs4803419), and metabolic parameters using linear regression in PLINK.[19] We did not correct for multiple comparisons. Genotyping was done in the Vanderbilt DNA Resources Core as described elsewhere.[20] Composite 516/983 or 15582/516/983 genotypes were as assigned as previously described.[20]

RESULTS

107 participants were recruited into the study. One participant with an efavirenz concentration below the limit of assay quantification was excluded from analyses for presumed non-adherence. Characteristics of the 106 evaluable participants are provided in **Table 1.** All participants were black Africans and most were women, reflecting the patient population at these clinics. More than 90% had been exposed to stavudine. All participants reported 100% adherence. Only 34 participants had plasma HIV-1 RNA data available within the previous 3 months. Metabolic abnormalities, as defined by NCEP III and ADA criteria, were common: hypercholesterolaemia was present in 40%, hypertriglyceridaemia in 26%, low HDL cholesterol in 49%, high LDL cholesterol in 42%, impaired fasting glucose in 24%, impaired glucose tolerance in 10%, and diabetes in 2%.

The median efavirenz concentration was 2.23 µg/mL (IQR 1.66 to 4.10 µg/mL). Median time after the last dose was 12.08 hours (IQR 11.62 to 12.78 hours). Relationships between plasma efavirenz concentrations and metabolic parameters are shown in **Figure 1.** There was a positive correlation between higher $\log_{10}$ transformed efavirenz concentrations and higher fasting total cholesterol, HDL cholesterol, LDL cholesterol concentrations, but not with triglycerides and 2-hour OGTT glucose concentrations. There was a non-significant correlation between plasma efavirenz concentrations and fasting glucose concentrations ($p = 0.073$).

In multivariate analyses that adjusted for age, sex, BMI, and duration on ART, $\log_{10}$ transformed efavirenz concentrations were independently associated with both lipid and glucose concentrations as displayed in **Table 2**. **Table 2** also shows the mean change in each metabolic parameter per doubling of efavirenz concentrations. In these multivariate analyses, advancing age was also independently associated with increasing cholesterol (total cholesterol, HDL and LDL cholesterol) and glucose (fasting and 2 hour), but not triglycerides (data not shown). When current stavudine use was included in the multivariate model with age, sex, BMI, and total duration on ART, similar associations between the $\log_{10}$ transformed efavirenz concentrations and both lipid and glucose concentrations were found (see Table, **Supplementary Digital Content 1,** a table that illustrates the multivariate regression analyses adjusting for age, sex, BMI, total duration on ART, and current stavudine use**).** Current stavudine use was not included in the final model because it was not a significant variable in all models (data not shown).

In the final multivariate regression model that included $\log_{10}$ transformed efavirenz concentrations, age, sex, BMI, and total duration on ART, the $\log_{10}$ transformed efavirenz concentrations explained 26% of interindividual variability for total cholesterol, 23% for HDL cholesterol, 20% for triglycerides, 19% for 2-hour glucose, 17% for fasting glucose, and 16% for LDL cholesterol.

Exploratory analysis examined relationships between *CYP2B6* genotypes and metabolic parameters among 57 participants with available genotype data. Allelic frequencies of *CYP2B6* 516G→T, 983T→C, and 15582C→T were 0.35, 0.08, and 0.08, respectively. Genotype frequencies are displayed in **Supplemental Digital Content 2** (see figure, Supplemental Figure 1, that illustrates a bar graph displaying *CYP2B6* genotype frequencies in 57 South African adults). There was a significant association between *CYP2B6* 516G→T and total cholesterol concentrations ($p = 0.048$) as shown in **Table 3**. In addition, for *CYP2B6* 516G→T, beta coefficients for total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides were generally consistent and in the same direction as beta coefficients for $\log_{10}$ plasma efavirenz concentrations for these parameters. There were trends toward significant associations between *CYP2B6* 516G→T and HDL cholesterol concentrations, and between composite 516/983 genotype and total and HDL cholesterol concentrations.

DISCUSSION

We investigated whether plasma efavirenz concentrations correlated with plasma lipid and/or glucose concentrations in HIV-infected South Africans. Higher plasma efavirenz concentrations were associated with significantly higher plasma fasting lipid concentrations and higher glucose concentrations in 106 HIV-infected South African adults receiving ART in multivariate analyses. In a subset of 57 participants with available *CYP2B6* genotype data, associations between slow metabolizer genotypes and metabolic profiles were generally consistent with associations based on measured efavirenz concentrations.

To our knowledge, this is the first report of positive associations between plasma efavirenz concentrations and LDL cholesterol, or glucose concentrations. Our findings are consistent with a previous study that showed an association between plasma efavirenz concentrations and fasting HDL cholesterol in 34 participants,[21] but disagree with a retrospective study that found no associations between plasma efavirenz concentrations and either fasting HDL cholesterol or triglycerides in 59 participants.[22]

The advent of ART has greatly reduced morbidity and mortality among HIV-infected patients.[23] However, the D:A:D (Data Collection on Adverse Events of Anti-HIV Drugs) study showed that patients treated with protease inhibitors or NNRTIs, alone or in combination, have elevated total cholesterol, after controlling for known risk factors such as age, BMI and sex.[24] Some of these abnormalities, notably LDL cholesterol and glucose, are associated with an increased risk of vascular disease in the general population.[25,26]

The prevalence of metabolic complications in our cohort was high (dysglycaemia 37% and dyslipidaemia 47%). The commonest dyslipidaemia we found was low HDL cholesterol in 49%, which

is lower than the 71% we found in ART-naïve patients drawn from the same clinics as the participants in our study (JA Dave, MBChB PhD, unpublished data, November 2015). However, high LDL cholesterol and high triglycerides, which were common in our study population, were rare in ART-naïve patients. High prevalence of both dyslipidaemia and dysglycaemia in patients on ART has also been reported from other African countries.[27-29]

There is considerable interindividual variability in plasma efavirenz exposure, approximately 34% of which is explained by three *CYP2B6* loss-of-function polymorphisms, *CYP2B6* 516G→T, 983T→C, and 15582C→T.[10,20] Among South Africans, minor allele frequencies of *CYP2B6* 516G→T, 983T→C, and 15582C→T have been reported to be 0.36, 0.07, and 0.09, respectively.[20] Both 516G→T and 983T→C are more frequent with African than with European ancestry. The *CYP2B6* 983T→C allele is found almost exclusively with African ancestry,[11] and has a somewhat greater effect on efavirenz concentrations per allele than does 516G→T, whereas 15582C→T has a much lesser effect than 516G→T. The lower prevalence of *CYP2B6* slow metabolizer genotypes with European ancestry may explain, in part, why some studies conducted largely in Europeans have not shown associations between efavirenz and cardiovascular risk.[30,31] In our study, the smaller number of participants with genotype data may have limited our ability to identify statistically significant genetic associations.

Our results have potential public health implications. Efavirenz is the preferred third drug, in combination with NRTIs, as first line therapy in resource-limited settings where the HIV-1 burden is greatest.[1] Efavirenz may be more likely to result in an increased risk of cardiovascular events among populations in whom *CYP2B6* slow metabolizer genotypes are prevalent. However, higher efavirenz concentrations were also associated with higher HDL cholesterol in our study, which has been associated with decreased risk of cardiovascular events. Newer agents such as rilpivirine and dolutegravir have little or no effect on lipids,[32,33] these drugs are currently not available in most low-middle income countries.

Our study has several limitations. Because it is a cross sectional study, we cannot compare changes from baseline in lipid or glucose concentrations in patients starting ART. The viral load data was determined by reviewing medical records and only 34 participants had viral load data recorded. Therefore, viral load was not included in our multivariate analyses. Our sample size is relatively small. We investigated associations with mid-dose interval efavirenz concentrations and metabolic parameters, but other pharmacokinetic parameters (e.g. area under the concentration-time curve) might better describe the relationships with metabolic profiles. Although participants recorded the time of last dose on the day before pharmacokinetic sampling, we cannot exclude incomplete adherence.

CONCLUSION

In conclusion, higher plasma efavirenz concentrations were associated with higher plasma lipid and glucose concentrations. However, larger cohort studies are needed to replicate these associations. Well-powered studies in Africa and other regions where efavirenz slow metabolizer genotypes are prevalent are needed to assess whether long-term efavirenz use is associated with increased risk of cardiovascular events.

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Author contributions

PZS participated in the study design, acquisition of data, data analysis and interpretation and drafted the manuscript. HMM participated in the study design, data interpretation, and critically revised the manuscript. PJS performed the analysis of the pharmacokinetic samples and helped to draft the manuscript. JAD participated in the study design, acquisition of data, and critically revised the manuscript. NSL participated in study design and critically revised the manuscript. GM participated in study design, data interpretation and critically revised the manuscript. DWH participated in the data analysis and interpretation and drafted the manuscript. All authors read and approved the final manuscript.

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Potential conflict of interest

DWH has been a consultant to Merck. The rest of the authors declare no support from any organization for the submitted work, and there are no other relationships or activities that could appear to have influenced the submitted work.

REFERENCES

1. World Health Organization. Consolidated guidelines on the use of antiretroviral drugs for treating and prenting HIV infection. 2013; http://www.who.int/hiv/pub/guidelines/arv2013/download/en/.

2. Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr.* 2011;57(4):284-289.

3. Fontas E, van Leth F, Sabin CA, et al. Lipid profiles in HIV-infected patients receiving combination antiretroviral therapy: are different antiretroviral drugs associated with different lipid profiles? *J Infect Dis.* 2004;189(6):1056-1074.

4. Tashima KT, Bausserman L, Alt EN, Aznar E, Flanigan TP. Lipid changes in patients initiating efavirenz- and indinavir-based antiretroviral regimens. *HIV Clin Trials.* 2003;4(1):29-36.

5. Williams P, Wu J, Cohn S, et al. Improvement in lipid profiles over 6 years of follow-up in adults with AIDS and immune reconstitution. *HIV Med.* 2009;10(5):290-301.

6. van Leth F, Phanuphak P, Stroes E, et al. Nevirapine and efavirenz elicit different changes in lipid profiles in antiretroviral-therapy-naive patients infected with HIV-1. *PLoS Med.* 2004;1(1):e19.

7. Rhoads MP, Lanigan J, Smith CJ, Lyall EG. Effect of specific ART drugs on lipid changes and the need for lipid management in children with HIV. *J Acquir Immune Defic Syndr.* 2011;57(5):404-412.

8. Carr A, Samaras K, Thorisdottir A, Kaufmann GR, Chisholm DJ, Cooper DA. Diagnosis, prediction, and natural course of HIV-1 protease-inhibitor-associated lipodystrophy, hyperlipidaemia, and diabetes mellitus: a cohort study. *Lancet.* 1999;353(9170):2093-2099.

9. Haas DW, Ribaudo HJ, Kim RB, et al. Pharmacogenetics of efavirenz and central nervous system side effects: an Adult AIDS Clinical Trials Group study. *AIDS.* 2004;18(18):2391-2400.

10. Holzinger ER, Grady B, Ritchie MD, et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. *Pharmacogenet Genomics.* 2012;22(12):858-867.

11. dbSNP. Short Genetic Variations. http://www.ncbi.nlm.nih.gov/projects/SNP/. .

12. Wang J, Sonnerborg A, Rane A, et al. Identification of a novel specific CYP2B6 allele in Africans causing impaired metabolism of the HIV drug efavirenz. *Pharmacogenet Genomics.* 2006;16(3):191-198.

13. Chou M, Bertrand J, Segeral O, et al. Population pharmacokinetic-pharmacogenetic study of nevirapine in HIV-infected Cambodian patients. *Antimicrob Agents Chemother.* 2010;54(10):4432-4439.

14. Sukasem C, Cressey TR, Prapaithong P, et al. Pharmacogenetic markers of CYP2B6 associated with efavirenz plasma concentrations in HIV-1 infected Thai adults. *Br J Clin Pharmacol.* 2012;74(6):1005-1012.

15. Ren Y, Nuttall JJ, Eley BS, et al. Effect of rifampicin on efavirenz pharmacokinetics in HIV-infected children with tuberculosis. *J Acquir Immune Defic Syndr.* 2009;50(5):439-443.

16. Association AD. Standards of medical care in diabetes--2013. *Diabetes Care.* 2013;36 Suppl 1:S11-66.

17. National Cholesterol Education Program (NCEP) Expert Panel on Detection E, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation.* 2002;106(25):3143-3421.

18. AIDS Clinical Trials Group. ACTG Adherence follow-up questionnaire. 2006; http://caps.ucsf.edu/resources/survey-instruments - 8.

19. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81(3):559-575.

20. Sinxadi PZ, Leger PD, McIlleron HM, et al. Pharmacogenetics of plasma efavirenz exposure in HIV-infected adults and children in South Africa. *Br J Clin Pharmacol.* 2015.

21. Pereira SA, Branco T, Corte-Real RM, et al. Long-term and concentration-dependent beneficial effect of efavirenz on HDL-cholesterol in HIV-infected patients. *Br J Clin Pharmacol.* 2006;61(5):601-604.

22. Autar RS, Boyd MA, Wit FW, et al. Relationships between drug exposure, changes in metabolic parameters and body fat in HIV-infected patients switched to a nucleoside sparing regimen. *Antivir Ther.* 2007;12(8):1265-1271.

342 23. Palella FJ, Jr., Delaney KM, Moorman AC, et al. Declining morbidity and mortality among
343 patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators.
344 *N Engl J Med.* 1998;338(13):853-860.
345 24. Friis-Moller N, Weber R, Reiss P, et al. Cardiovascular disease risk factors in HIV patients--
346 association with antiretroviral therapy. Results from the DAD study. *AIDS.* 2003;17(8):1179-1193.
347 25. Friis-Moller N, Thiebaut R, Reiss P, et al. Predicting the risk of cardiovascular disease in
348 HIV-infected patients: the data collection on adverse effects of anti-HIV drugs study. *Eur J Cardiovasc*
349 *Prev Rehabil.* 2010;17(5):491-501.
350 26. Anand SS, Islam S, Rosengren A, et al. Risk factors for myocardial infarction in women and
351 men: insights from the INTERHEART study. *Eur Heart J.* 2008;29(7):932-940.
352 27. Zannou DM, Denoeud L, Lacombe K, et al. Incidence of lipodystrophy and metabolic
353 disorders in patients starting non-nucleoside reverse transcriptase inhibitors in Benin. *Antivir Ther.*
354 2009;14(3):371-380.
355 28. Mutimura E, Stewart A, Rheeder P, Crowther NJ. Metabolic function and the prevalence of
356 lipodystrophy in a population of HIV-infected African subjects receiving highly active antiretroviral
357 therapy. *J Acquir Immune Defic Syndr.* 2007;46(4):451-455.
358 29. Omech B, Sempa J, Castelnuovo B, et al. Prevalence of HIV-Associated Metabolic
359 Abnormalities among Patients Taking First-Line Antiretroviral Therapy in Uganda. *ISRN AIDS.*
360 2012;2012:960178.
361 30. Samaras K, Wand H, Law M, Emery S, Cooper D, Carr A. Prevalence of metabolic syndrome
362 in HIV-infected patients receiving highly active antiretroviral therapy using International Diabetes
363 Foundation and Adult Treatment Panel III criteria: associations with insulin resistance, disturbed body
364 fat compartmentalization, elevated C-reactive protein, and [corrected] hypoadiponectinemia. *Diabetes*
365 *Care.* 2007;30(1):113-119.
366 31. Lang S, Mary-Krause M, Cotte L, et al. Impact of individual antiretroviral drugs on the risk of
367 myocardial infarction in human immunodeficiency virus-infected patients: a case-control study nested
368 within the French Hospital Database on HIV ANRS cohort CO4. *Arch Intern Med.* 2010;170(14):1228-
369 1238.

370 32. Tebas P, Sension M, Arribas J, et al. Lipid levels and changes in body fat distribution in
371 treatment-naive, HIV-1-Infected adults treated with rilpivirine or Efavirenz for 96 weeks in the ECHO
372 and THRIVE trials. *Clin Infect Dis.* 2014;59(3):425-434.
373 33. Quercia R, Roberts J, Martin-Carpenter L, Zala C. Comparative changes of lipid levels in
374 treatment-naive, HIV-1-infected adults treated with dolutegravir vs. efavirenz, raltegravir, and
375 ritonavir-boosted darunavir-based regimens over 48 weeks. *Clin Drug Investig.* 2015;35(3):211-219.

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FIGURE LEGEND

Figure 1. Scatter plots of log_{10} transformed plasma efavirenz concentrations (µg/mL) and the lipid and glucose concentrations in 106 participants. The x-axes represent log_{10} transformed plasma efavirenz concentrations. The y-axes represent concentrations of each metabolic parameter [(a): fasting total cholesterol, (b): fasting triglycerides, (c): fasting HDL cholesterol, (d): fasting LDL cholesterol, (e): fasting glucose, and (f): glucose 2 hours post oral glucose tolerance test]. Each black marker denotes an individual lipid or glucose concentration plotted against the plasma efavirenz concentrations in 106 participants. The solid black line indicates the regression line, with the 95% confidence interval shown in dotted lines. The equations for the regression line (with the slope and y intercept), correlation determinants (R^2), and p-values are shown on each plot. LDL= low density lipoprotein, HDL= high density lipoprotein

SUPPLEMENTAL DIGITAL CONTENT LEGEND

Supplemental Digital Content 1. Table that illustrates the multivariate regression analyses adjusting for age, sex, body mass index, total duration on ART, and current stavudine use.

Supplemental Digital Content 2. Figure that illustrates a bar graph displaying *CYP2B6* genotype frequencies in 57 South African adults.

Table 1. Study participant characteristics

Variable	Median (IQR) or n/N(%)	N
Age (years)	38 (31 to 45)	106
Male: Female	29:77	106
Weight (kg)	69 (60 to 78)	106
Body mass index (kg/m^2)	26 (23 to 30)	106
Waist: hip ratio	0.89 (0.85 to 0.93)	106
Blood pressure (mmHg)		104
Systolic	113 (101 to 125)	
Diastolic	72 (64 to 81)	
CD4+ T-cell count (cells/ mm^3)		
Pre-ART	94 (43 to 153)	89
Current	316 (243 to 503)	102
Plasma HIV-1 RNA <400 copies/mL (%)	28/34 (82%)	34
Total duration on ART (months)	18 (10 to 27)	106
Concurrent ART n/N (%)		106
Zidovudine/lamivudine	31/106 (29%)	
Stavudine/lamivudine	75/106 (71%)	
Any stavudine exposure	99/106 (93%)	106
Metabolic parameters (mmol/L)		106
Fasting total cholesterol	4.76 (3.95 to 5.65)	
Fasting LDL cholesterol	3.08 (2.32 to 3.77)	
Fasting HDL cholesterol	1.05 (0.81 to 1.30)	
Fasting triglycerides	1.26 (1.01 to 1.79)	
Fasting glucose	5.20 (4.80 to 5.80)	
2-hour post OGTT glucose	5.70 (4.80 to 6.80)	

IQR= interquartile range, n/N = number of participants with variable of interest/total number of the study population, ART= antiretroviral therapy, HIV=Human Immunodeficiency Virus, RNA= ribonucleic acid, LDL= low-density lipoprotein, HDL= high-density lipoprotein, OGTT= oral glucose tolerance test.

Scatter plots of log_{10} transformed plasma efavirenz concentrations (µg/mL) and the lipid and glucose concentrations in 106 participants

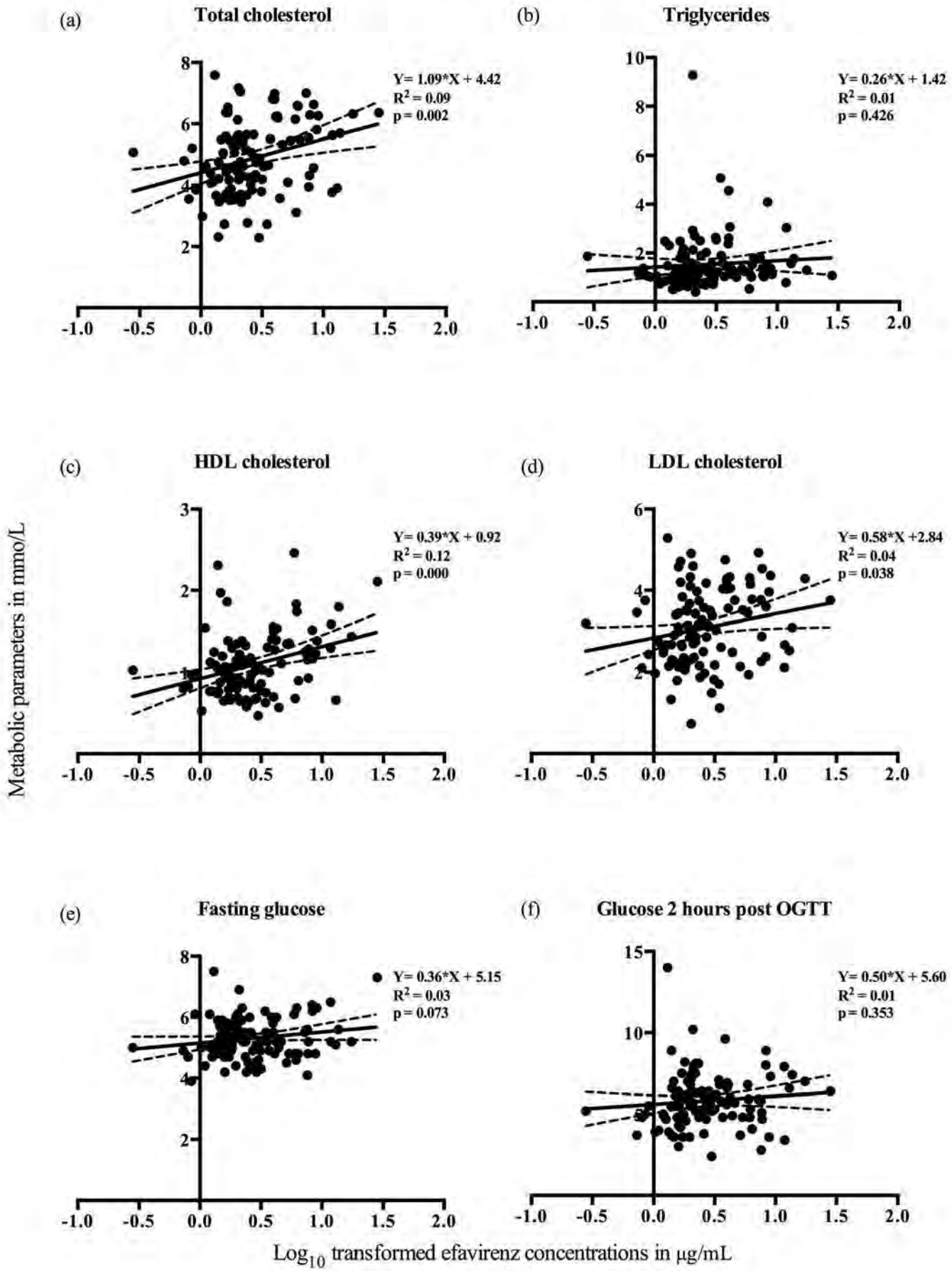

Table 2. Multivariate linear regression analyses (each adjusted for age, body mass index and total duration on ART) between log_{10} transformed efavirenz mid-dosing interval concentrations and each metabolic parameter (N=106)

Outcome variable	β (95%CI)	*Mean change (95% CI) per doubling of efavirenz concentrations (mmol/L)	p-value
Total cholesterol	1.34 (0.73 to 1.95)	0.40 (0.22 to 0.59)	0.000
LDL cholesterol	0.62 (0.14 to 1.01)	0.19 (0.04 to 0.30)	0.012
HDL cholesterol	0.46 (0.24 to 0.67)	0.14 (0.07 to 0.20)	0.000
Triglycerides	0.58 (0.09 to 1.08)	0.17 (0.03 to 0.33)	0.022
Fasting glucose	0.60 (0.11 to 1.10)	0.18 (0.03 to 0.33)	0.017
2-hour glucose	1.14 (0.28 to 2.00)	0.34 (0.08 to 0.60)	0.010

ART= antiretroviral therapy, N = total number of the study population, LDL= low-density lipoprotein, HDL = high-density lipoprotein. *Mean change (95%CI) in lipid and glucose concentrations (in mmol/L) with doubling of efavirenz concentrations. The mean changes are calculated using the formula Y= β*log10 (2), where β is the beta coefficient for the log_{10} transformed efavirenz concentrations as the independent variable in linear regression analyses for the various metabolic outcomes.

2

PLASMA EFAVIRENZ CONCENTRATIONS ARE ASSOCIATED WITH LIPID AND GLUCOSE CONCENTRATIONS

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Supplementa1 Table 1. Multivariate linear regression analyses (each adjusted for age, body mass index, total duration on ART and current stavudine use) between log_{10} transformed efavirenz mid-dosing interval concentrations and each metabolic parameter (N=106)

Outcome variable	β (95%CI)	*Mean change (95% CI) per doubling of efavirenz concentrations (mmol/L)	p-value
Total cholesterol	1.31 (0.70 to 1.93)	0.39 (0.21 to 0.58)	0.000
LDL cholesterol	0.60 (0.12 to 1.08)	0.18 (0.04 to 0.32)	0.015
HDL cholesterol	0.45 (0.23 to 0.67)	0.14 (0.07 to 0.20)	0.000
Triglycerides	0.59 (0.09 to 1.09)	0.18 (0.03 to 0.33)	0.020
Fasting glucose	0.59 (0.08 to 1.09)	0.18 (0.02 to 0.33)	0.023
2-hour glucose	1.10 (0.21 to 1.99)	0.33 (0.06 to 0.60)	0.016

ART= antiretroviral therapy, N = total number of the study population, LDL= low-density lipoprotein, HDL = high-density lipoprotein. *Mean change (95%CI) in lipid and glucose concentrations (in mmol/L) with doubling of efavirenz concentrations. The mean changes are calculated using the formula Y= β*log10 (2), where β is the beta coefficient for the log_{10} transformed efavirenz concentrations as the independent variable in linear regression analyses for the various metabolic outcomes.

PLASMA EFAVIRENZ CONCENTRATIONS ARE ASSOCIATED WITH LIPID AND GLUCOSE CONCENTRATIONS

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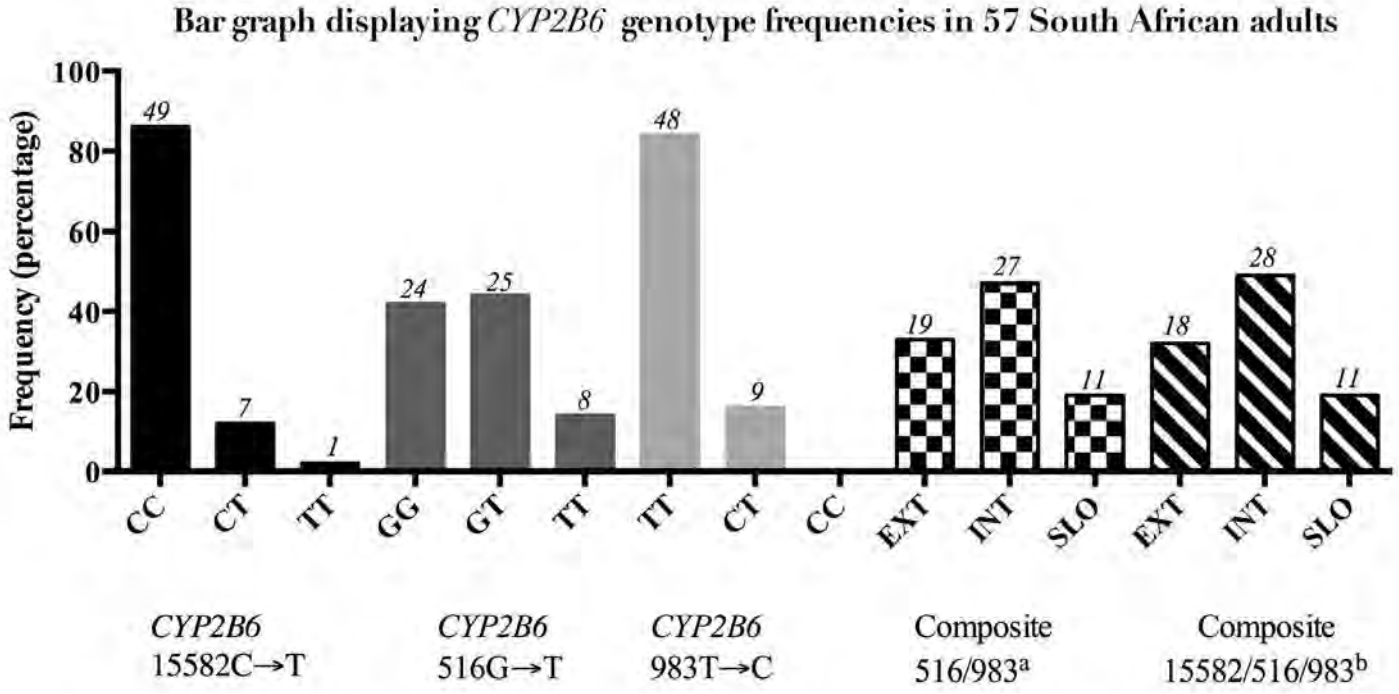

Supplemental Figure 1. Bar graph displaying *CYP2B6* genotype frequencies in 57 South African adults. The x-axis represents CYP2B6 genotypes: 15582C→T (solid black), *CYP2B6* 516G→T (dark grey), 983T→C (light grey), composite 516/983 (black and white squares), and composite 15582/516/983 (black and white horizontal lines) genotypes. The composite genotypes were categorized into three levels; extensive metabolizer (EXT), intermediate metabolizer (INT) and slow metabolizer (SLO). The y-axis represents genotype frequencies in percentages. The number in italics at the top of each bar displays the absolute count. a Composite 516/983 were assigned as follows: EXT (516GG-983TT); INT (516GT-983TT or 516GG-983CT); and SLO (516TT-983TT, 516GT-983CT). b Composite 15582/516/983 were assigned as follows: EXT (15582CC-516GG-983TT or 15582CT-516GG-983TT); INT (15582TT-516GG-983TT, 15582CC-516GT-983TT, 15582CC-516GG-983CT, 15582CT-516GT-983TT, or 15582CT-516GG-983CT); and SLO (15582CC-516TT-983TT, 15582CC-516GT-983CT).

Table 3. Exploratory analyses between known *CYP2B6* polymorphisms and metabolic parameters in 57 participants

	Chromosome 19 *CYP2B6* polymorphisms									
Outcome variable (mmol/L)	***CYP2B6* 516 G→T (rs3745274)**		***CYP2B6* 983 T→C (rs28399499)**		***CYP2B6* 15582 C→T (rs4803419)**		**Composite SNP 516/983**		**Composite SNP 15582/516/983**	
	β(95%CI)	**p**	**β(95%CI)**	**p**	**β(95%CI)**	**p**	**β(95%CI)**	**p**	**β(95%CI)**	**p**
Total cholesterol	0.46 (0.02 to 0.90)	**0.048**	-0.22 (-1.01 to 0.66)	0.634	-0.02 (-1.01 to 0.66)	0.965	0.39 (-0.05 to 0.83)	0.091	0.33 (-0.12 to 0.78)	0.160
LDL cholesterol	0.21 (-0.14 to 0.56)	0.243	-0.08 (-0.75 to 0.60)	0.828	0.20 (-0.39 to 0.80)	0.506	0.18 (-0.16 to 0.25)	0.300	0.15 (-0.20 to 0.50)	0.413
HDL cholesterol	0.13 (-0.01 to 0.27	0.065	-0.01 (-0.31 to 0.24)	0.799	-0.07 (-0.31 to 0.17)	0.556	0.12 (-0.02 to 0.25)	0.093	0.10 (-0.03 to 0.24)	0.150
Triglycerides	0.26 (-0.04 to 0.56)	0.104	-0.23 (-0.82 to 0.37)	0.456	-0.33 (-0.85 to 0.20)	0.228	0.19 (-0.11 to 0.49)	0.228	0.17 (-0.14 to 0.48)	0.282
Fasting glucose	0.36 (-0.17 to 0.34)	0.495	-0.21 (-0.72 to 0.29)	0.412	0.03 (-0.42 to 0.48)	0.896	0.03 (-0.23 to 0.29)	0.802	-0.01 (-0.27 to 0.26)	0.962
2 hour glucose	-0.04 (-0.71 to 0.64)	0.919	-0.59 (-1.87 to 0.67)	0.373	-0.18 (-1.13 to 0.97)	0.755	-0.19 (-0.85 to 0.47)	0.579	-0.22 (-0.89 to 0.45)	0.525

2

STROBE Statement

Checklist of items that should be included in reports of observational studies

Section/Topic	Item No	Recommendation	Reported on Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	3
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	3
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	5
Data sources/measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	5
Bias	9	Describe any efforts to address potential sources of bias	5
Study size	10	Explain how the study size was arrived at	6
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6
		(*b*) Describe any methods used to examine subgroups and interactions	6
		(*c*) Explain how missing data were addressed	5
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	

	Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
	(*e*) Describe any sensitivity analyses	5

Section/Topic	Item No	Recommendation	Reported on Page No
ults			
icipants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	6
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
criptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	6, Table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
come data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	6
n results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Figure 1 Table 2, 3
		(*b*) Report category boundaries when continuous variables were categorized	5
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
er analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	7
cussion			
results	18	Summarise key results with reference to study objectives	7
itations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
rpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	8
eralisability	21	Discuss the generalisability (external validity) of the study results	8
er Information1			
ding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies. **Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

CHAPTER 7

Conclusions

Chapter 7

Conclusions

Improved access to ART has led to the decline in HIV associated morbidity and mortality. However, cumulative exposure to antiretroviral therapy (ART) has been associated with metabolic adverse effects such as dyslipidaemia, insulin resistance, dysglycaemia, central fat accumulation, peripheral fat loss (lipoatrophy), and peripheral neuropathy.

This thesis collection of studies nested within a cross-sectional study that enrolled ambulant HIV infected participants in Cape Town between 2007-2009. The details of the main study are described in the introduction and elsewhere.[1] The aim of the main study was to determine the prevalence of DSP, dysglycaemia, dyslipidaemia, hyperlactataemia and lipodystrophy. We collected clinical and laboratory data from HIV infected patients on ART from Cape Town. We sequenced the mitochondrial genome and determined African mtDNA haplogroups. We genotyped 241 polymorphisms in genes potentially relevant to efavirenz metabolism and transport. We measured steady state efavirenz and lopinavir concentrations and used regression analyses to determine associations with metabolic parameters.

This thesis presents studies that investigated associations between plasma lopinavir concentrations and lipid and glucose concentrations (Chapter 3), explored associations of mitochondrial DNA (mtDNA) haplogroups and ART related metabolic complications (chapter 4), characterized relationships between genetic polymorphisms and plasma efavirenz concentrations (chapter 5), and investigated associations between plasma efavirenz concentrations and lipid and glucose concentrations (chapter 6) in HIV-infected Black South Africans.

The study in **Chapter 3** reported data on 84 black South African HIV-infected adults treated with ritonavir-boosted lopinavir for a median duration of 19 months, had lopinavir trough

concentrations measured and the associations with lipids and glucose concentrations were investigated. To our knowledge, this is the first study to investigate the association between lopinavir concentrations and serum lipids or glucose concentrations in a South African population. There was a high prevalence of dyslipidaemia (29%) and dysglycaemia (42%). Despite that, there were no significant associations between plasma lopinavir concentrations and lipid or glucose concentrations. There was also no significant association between the lopinavir concentrations above the median, and hypercholesterolaemia, hypertriglyceridaemia or dysglycaemia. The median lopinavir concentration was 8 µg/mL, which is higher than reported elsewhere,[2,3] but comparable to the trough concentrations after observed doses in a study conducted by our group from the same community.[4] Our findings are in contrast with findings from a small study conducted in 19 patients, which reported that lopinavir trough concentrations were higher in three patients experiencing grade 3 or 4 lipid elevations.[5] A second larger study (n=126) found that patients with fasting triglyceride concentrations above the median had higher lopinavir trough concentrations, but no correlation was found between lopinavir and cholesterol concentrations.[6] Four other studies reported findings similar to ours, with no association between lopinavir and lipids.[3,7-9] In one of these studies, the investigators also reported no association between lopinavir concentrations and glucose concentrations.[8]

The study reported in **Chapter 4** explored associations between African mtDNA haplogroups and ART-related complications in a cohort of HIV-infected South African adults. In this study, L3e1 subhaplogroup was significantly associated with hypertriglyceridemia independent of lopinavir/ritonavir exposure. This is the first report to link an African mtDNA variant to hypertriglyceridemia. We found no associations between mtDNA haplogroups and other metabolic complications, including hypercholesterolemia, dysglycemia, lipoatrophy, or DSP.

We also described the frequencies of the mitochondrial haplogroups in our South African cohort recruited from two Cape Town clinics, the majority of which belonged to the L0 haplogroup.

There are limited data regarding the distribution of the mitochondrial haplogroups in the South African population. However, the distribution of mitochondrial haplogroups we found was similar to a recently published South African study conducted in 71 children.[10] None of the participants analyzed belonged to the European mitochondrial haplogroups.

The association between L3e1 subhaplogroup and hypertriglyceridemia was independent of LPV/r use, which is a well-documented cause of elevated triglycerides.[11] In a recent study of 174 non-Hispanic white HIV-infected clinical trial participants in the United States, the European mtDNA haplogroup I was associated with a decrease in triglycerides over 96 weeks of ART when compared with non-I mtDNA haplogroups.[12] To the best of our knowledge, the association between African mtDNA haplogroups and hypertriglyceridemia has not previously been studied.

An association between an mtDNA polymorphism and plasma triglycerides was previously reported in a Canadian cohort.[13] Variation at mtDNA position 16517 was associated with significantly higher fasting plasma triglyceride concentrations in this population. These data support the hypothesis that mitochondrial DNA variation may influence fatty acid and lipid metabolism. Fatty acids derived from plasma triglycerides are precursors of acyl CoA, which is used in the mitochondrial ß oxidation cycle of fatty acid metabolism. Genetic variation affecting mitochondrial function might also affect the utilization of fatty acid in ß oxidation.[13] Differences in cellular utilization may then alter the demand of fatty acids from triglycerides, which may influence plasma concentrations.[13] However, the exact mechanism is still unclear.

Finally, we found no association between African mtDNA haplogroups and DSP. A study conducted in non-Hispanic black participants from ACTG study 384 reported an association between the African mtDNA L1c and increased susceptibility to peripheral neuropathy during NRTI treatment.[14] As discussed above, the L1 haplogroup was found in only three participants in our cohort and they were excluded from our analyses, so we were unable to replicate this reported

association. Here, DSP was frequent and no association was observed with a particular haplogroup. We were also unable to adjust for the effect of d-drugs, an important cause of DSP, as >90% of participants had been exposed to stavudine and/or didanosine. All participants were examined by either of two trained clinicians, and DSP was more rigorously defined compared with many other reports. The prevalence of DSP in our cohort was high (66%), compared with that reported in the ACTG study (33%).[14] Since the publication of this study, a study conducted in Malawians reported an associations between peripheral neuropathy and L0a2 (increase susceptibility) and L2a (protective). The discrepant results can be explained by differences in the haplogroup frequencies between the two African countries as well as the different ways of phenotype ascertainment resulting in a remarkable difference in DSP prevalence (25% vs 66% in our study). Kampira et al based their case definition of DSP on symptoms, which may misclassify subjects with asymptomatic DSP and controls, while our study defined DSP after neurological examination and symptoms.

In **Chapter 5**, the pharmacogenetics study of efavirenz exposure in 113 HIV-infected black South African adults and children is reported. Efavirenz is one of the most extensively prescribed medications worldwide for HIV-1 infection, and multiple previous studies have associated *CYP2B6* 516 G→T, and *CYP2B6* 983 T→C with increased plasma efavirenz concentrations.[15] Our study replicated these associations in Black South Africans. In addition, we show for the first time that *CYP2B6* 15582C→T is also associated with plasma efavirenz concentrations in Black South Africans, which had previously only been reported for efavirenz in one study from the United States.[16] In univariate analyses, a model that included composite genotype (*CYP2B6* 516/983/15582) best predicted efavirenz concentrations. These associations were consistent in adults and children. An association between *CYP2B6* 15582C→T and slower plasma drug clearance has also been reported among Cambodians for the CYP2B6 substrate nevirapine,[17] further supporting the validity of our finding. We did not find significant associations beyond

CYP2B6 516G→T, 983T→C, and 15582C→T. Although one analysis suggested an association with *NR1I2* rs9847782, this was no longer apparent after excluding one outlier, suggesting a spurious association. Our study also provides information regarding minor allele frequencies for *ABCB1*, *CYP2A6*, *CYP2B6*, *CYP3A4*, *NR1I2* and *NR1I3* polymorphisms in a South African population.

We observed lower efavirenz concentrations in children, which are probably explained by the faster rate of drug metabolism in children compared with adults.[18] High prevalence of low efavirenz concentrations in children have also been attributed to the current weight based WHO guidelines, and have been observed in a South African population similar to ours.[19] We assessed associations between polymorphisms and efavirenz concentrations separately in adults and children. In both children and adults, *CYP2B6* 516G→T had the strongest overall association with efavirenz concentrations, although *CYP2B6* 983T→C had the greatest effect size per allele. The association with *CYP2B6* 15582C→T could not be demonstrated in adults and children analyzed separately, possibly due to smaller samples sizes.

In this study, minor allele frequencies of *CYP2B6* 516G→T, 983T→C, and 15882C→T were 0.36, 0.07, and 0.09, respectively. In the multivariate regression model including all three polymorphisms, *CYP2B6* 983T→C had the greatest magnitude of effect on $\log_{10}$ efavirenz concentrations (β=0.38 for 983T→C, β=0.27 for 516G→T, β=0.06 for 15882C→T), but due to lower frequency only explained approximately 8% of variance in univariate analysis, versus 19% for 516G→T, and negligible effect for 15882C→T. In sensitivity analyses that excluded two individuals with extreme outlier efavirenz concentrations, the variance explained by all three polymorphisms increased from 34% to 45%.

In **chapter 6**, we investigated whether plasma efavirenz concentrations correlated with plasma lipid and/or glucose concentrations in HIV-infected South Africans. Higher plasma efavirenz

concentrations were significantly associated with higher plasma fasting lipid concentrations and higher glucose concentrations in 106 HIV-infected South African adults receiving ART in multivariate analyses. In a subset of 57 participants with available *CYP2B6* genotype data, associations between slow metabolizer genotypes and metabolic profiles were generally consistent with associations based on measured efavirenz concentrations. To our knowledge, this is the first report of positive associations between plasma efavirenz concentrations and LDL cholesterol, or glucose concentrations. Our findings are consistent with a previous study that showed an association between plasma efavirenz concentrations and fasting HDL cholesterol in 34 participants,[20] but disagree with studies that found no associations between plasma efavirenz concentrations and either fasting HDL cholesterol.[21,22]

In summary, the four studies have significantly contributed to the field of HIV research in the following ways:

First, we investigated associations between lopinavir and efavirenz drug concentrations and metabolic complications. This is the first time these associations, or lack thereof, have been described in an African population from Sub-Saharan region, the region hardest hit by the HIV burden. In children aged ≥3 years and adults, the World Health Organization (WHO) recommends combination ART consisting of a nucleoside (nucleotide) reverse transcriptase inhibitor (NRTI) backbone with either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a ritonavir-boosted protease inhibitor (PI) for first and second line regimens.[23] The finding that efavirenz concentrations positively correlated with lipids and glucose could increase long term risk of cardiovascular disease, which important public health implications for screening for risk factors or for selecting safer antiretrovirals.

Second, we describe the mitochondrial DNA haplogroup frequencies in Black South Africans. This highlights the differences in the most prevalent haplogroups between African-Americans and South Africans, or between South Africans and Malawians.[14,24] For example, in African-

Americans subhaplogroup L1c was reported to increase susceptibility to peripheral neuropathy.[14] In 171 participants enrolled in our study, only 3 people belonged to haplogroup L1 and were excluded from analyses. More importantly, we describe a novel association between African mitochondrial subhaplogroup L3e1 and hypertriglyceridaemia. This finding was independent of the well-described association between lopinavir and hypertriglyceridaemia. This finding has yet to be replicated. We found no associations between mtDNA haplogroup and other features of mitochondrial toxicity due to NRTIs (DSP, lipoatrophy and lactate)

Third, we described minor allele frequencies of 241 single nucleotide polymorphisms in genes relevant to efavirenz metabolism and drug transporters. We provide evidence to show that CYP2B6 15582C→T is associated with plasma efavirenz concentrations in Black South Africans, in addition to CYP2B6 516G→T and 983T→C. We show that genetic associations are consistent in adults and children. We also found no additional associations with plasma efavirenz concentrations beyond these *CYP2B6* polymorphisms. As efavirenz is still recommended as first line regimen for children aged ≥3 years and adults, in Sub-Saharan Africa, where the HIV burden is the highest, and both *CYP2B6* 516G→T and 983T→C alleles are more frequent, these patients are more likely to have higher efavirenz concentrations, and are at increased risk of adverse events.

Fourth, we explored associations in a subset of participants with *CYP2B6* 516/983/15582 composite genotype data and lipids and glucose concentrations. We found that in *CYP2B6* slow metabolizer, lipids and glucose concentrations tended to be higher. This needs further exploration in larger studies.

Limitations of the studies

The findings from these studies need to be interpreted within the context of their limitations. For the studies investigating associations between drug concentration and lipids and glucose (**chapters 3 and 6**) the limitations are as follows: First, the cross sectional study design limited the ability to compare baseline lipid or glucose concentrations prior to ART start. Second, patients with known diabetes or dyslipidaemia were excluded from the study. However, this is unlikely to have made a difference as only a small number were excluded. Third, the timing of the doses of lopinavir or efavirenz was not observed. To minimize recall bias, participants were requested to record the time of last dose on the appointment card for the day before pharmacokinetic sampling. Adherence was self-reported. Therefore, incomplete adherence cannot be excluded, which could have important effects on the observed concentrations. Fourth, we used lopinavir trough and efavirenz mid-dosing interval concentrations and not the area under the curve, the pharmacokinetic parameter that better describes the drug exposure. Fifth, viral load was missing in the majority of participants and therefore could not be used as a covariate in multivariate analyses. Sixth, the sample size may have limited our ability to find associations between lopinavir and lipids and glucose. However, it should be noted that our study was larger than most other studies that explored these associations.

The study reported in chapter 4 that investigated an association between African mtDNA and metatabolic complications had similar limitations to those decribed above. The design was cross-sectional, with participants of different HIV disease stages and different ART durations. We excluded ART-naive participants and participants with current opportunistic infections, known hepatic or renal disease, or with known dyslipidemia (or taking lipid-lowering therapy) or dysglycemia (or taking antidiabetic drugs). Viral load data were missing in the majority of participants, and were therefore not included in the analysis as a covariate. The sample size was relatively small and we did not formally adjust for multiple comparisons. However, when

considering the five mtDNA subhaplogroups we analyzed, only the unadjusted analyses between subhaplogroup L3e1 and triglycerides concentrations (Wilcoxon $p=0.003$), or hypertriglyceridemia ($p=0.007$) remained significant when our level of significance was corrected to $p<0.01$.

The study in **chapter 5** investigated 241 single nucleotide polymporhisms in 113 participants. Sample size limited our ability to extensively identify novel genetic associations with efavirenz concentrations. We pooled analyses between adults and children to improve power, and associations in this pooled analysis were similar to those in adults and children analyzed separately. In addition, because the dose of efavirenz was not observed, we could not be certain of dose-sampling times.

Implications for HIV research

In **chapter 3,** we did not find associations between lopinavir concentrations and lipids and glucose concentrations. Larger prospective studies are needed to establish whether the association exists between lopinavir concentrations and increasing lipids or glucose concentrations. We found high lopinavir concentrations in our cohort, a finding similar to a study conducted from the same population.[4] Lopinavir has large interindividual pharmacokinetic variability, at least part of which can be explained by age, sex, body weight, drug-drug interactions, liver disease, pregnancy and poor adherence.[25] The remaining variability is attributed to host genetic factors. Gene-gene interactions between genetic polymorphisms that influence lopinavir pharmacokinetics and the development of dysglycaemia or dyslipidaemia should be explored.

In **chapter 4**, we observed a novel association between African mtDNA subhaplogroup L3e1 and hypertriglyceridemia. We did not find associations between African mtDNA haplogroups and the other ART-related complications studied, but we may have been underpowered to identify

smaller associations. Larger studies in sub-Saharan Africa, which has the highest HIV burden in the world but few pharmacogenomic studies, are needed to confirm the association we found between African mtDNA subhaplogroup L3e1 and hypertriglyceridemia, and to explore associations between mtDNA haplogroups and other HIV- and ART-related complications. If the association between L3e1 and hypertriglyceridemia is confirmed, functional studies are needed to unravel the mechanism by which this subhaplogroup may increase triglycerides.

The study in **chapter 5** improves the understanding of genetic determinants of efavirenz plasma exposure in an African population, including adults and children. Studies of associations between efavirenz concentrations and polymorphisms in African populations should consider *CYP2B6* 516G→T and 983T→C, and ideally also 15582C→T.

The study in **chapter 6** showed that higher plasma efavirenz concentrations were associated with higher plasma lipid and glucose concentrations. Larger prospective cohort studies are needed to replicate these associations. Well-powered studies in Africa and other regions where efavirenz slow metabolizer genotypes are prevalent are needed to assess whether long-term efavirenz use is associated with increased risk of cardiovascular events.

Implications for clinical practice

Our findings improve the understanding of genetic determinants of efavirenz plasma exposure in an African population, and provide new insights into host factors associated with ART related metabolic complications. Some of our study findings have potential public health implications.

Efavirenz is the preferred third drug, in combination with NRTIs, as first line therapy in resource-limited settings where the HIV-1 burden is greatest.[23] Efavirenz may be more likely to result in an increased risk of cardiovascular events among populations in whom *CYP2B6* slow metabolizer genotypes are prevalent. However, higher efavirenz concentrations were also associated with

higher HDL cholesterol in our study, which has been associated with decreased risk of cardiovascular events. Newer agents such as rilpivirine and dolutegravir have little or no effect on lipids,[26,27] these drugs are currently not available in most low-middle income countries, but there is increasing interest in using them, as they are better tolerated than efavirenz.

In Sub-Saharan Africa, where the *CYP2B6* slow metabolizer genotypes are prevalent, and efavirenz is likely to continue to be prescribed as the preferred third drug in the medium, reducing the doses from 600mg to 400mg in patients is likely to lead to reduced adverse events and additionally, be cost saving. Monitoring and managing other risk factors for cardiovascular disease is vital.

The clinical utility of efavirenz genotyping or mitochondrial sequencing still needs further work and cannot be recommended based on our findings. Our findings do not support therapeutic drug monitoring of lopinavir for the prevention of metabolic complications. The clinical utility and cost-effectiveness of monitoring efavirenz concentrations in populations where *CYP2B6* slow metabolizer genotypes are prevalent still needs to be determined.

Bibliography and literature cited

1. Dave JA, Lambert EV, Badri M, West S, Maartens G, Levitt NS. Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr.* 2011;57(4):284-289.
2. Hurst M, Faulds D. Lopinavir. *Drugs.* 2000;60(6):1371-1379; discussion 1380-1371.
3. Bierman WF, van Vonderen MG, Veldkamp AI, et al. The lopinavir/ritonavir-associated rise in lipids is not related to lopinavir or ritonavir plasma concentration. *Antivir Ther.* 2011;16(5):647-655.
4. Decloedt EH, McIlleron H, Smith P, Merry C, Orrell C, Maartens G. Pharmacokinetics of lopinavir in HIV-infected adults receiving rifampin with adjusted doses of lopinavir-ritonavir tablets. *Antimicrob Agents Chemother.* 2011;55(7):3195-3200.
5. Gutierrez F, Padilla S, Navarro A, et al. Lopinavir plasma concentrations and changes in lipid levels during salvage therapy with lopinavir/ritonavir-containing regimens. *J Acquir Immune Defic Syndr.* 2003;33(5):594-600.
6. Gonzalez de Requena D, Blanco F, Garcia-Benayas T, Jimenez-Nacher I, Gonzalez-Lahoz J, Soriano V. Correlation between lopinavir plasma levels and lipid abnormalities in patients taking lopinavir/ritonavir. *AIDS patient care and STDs.* 2003;17(9):443-445.
7. Rhee MS, Hellinger JA, Sheble-Hall S, Cohen CJ, Greenblatt DJ. Relationship between plasma protease inhibitor concentrations and lipid elevations in HIV patients on a double-boosted protease inhibitor regimen (saquinavir/lopinavir/ritonavir). *J Clin Pharmacol.* 2010;50(4):392-400.
8. Leon A, Martinez E, Sarasa M, et al. Impact of steady-state lopinavir plasma levels on plasma lipids and body composition after 24 weeks of lopinavir/ritonavir-containing therapy free of thymidine analogues. *J Antimicrob Chemother.* 2007;60(4):824-830.

9. Ter Hofstede HJ, Koopmans PP, Burger DM, et al. Lopinavir Plasma Concentrations and Serum Lipids in Therapy Naïve HIV-Patients: A Sub Analysis of the FREE Study. 2012.
10. van der Walt EM, Smuts I, Taylor RW, et al. Characterization of mtDNA variation in a cohort of South African paediatric patients with mitochondrial disease. *European journal of human genetics : EJHG.* 2012;20(6):650-656.
11. Montes ML, Pulido F, Barros C, et al. Lipid disorders in antiretroviral-naive patients treated with lopinavir/ritonavir-based HAART: frequency, characterization and risk factors. *J Antimicrob Chemother.* 2005;55(5):800-804.
12. Hulgan T, Haubrich R, Riddler SA, et al. European mitochondrial DNA haplogroups and metabolic changes during antiretroviral therapy in AIDS Clinical Trials Group Study A5142. *AIDS.* 2011;25(1):37-47.
13. Hegele RA, Zinman B, Hanley AJ, Harris S, Connelly PW. A common mtDNA polymorphism associated with variation in plasma triglyceride concentration. *Am J Hum Genet.* 1997;60(6):1552-1555.
14. Canter JA, Robbins GK, Selph D, et al. African mitochondrial DNA subhaplogroups and peripheral neuropathy during antiretroviral therapy. *J Infect Dis.* 2010;201(11):1703-1707.
15. Aung AK, Haas DW, Hulgan T, Phillips EJ. Pharmacogenomics of antimicrobial agents. *Pharmacogenomics.* 2014;15(15):1903-1930.
16. Holzinger ER, Grady B, Ritchie MD, et al. Genome-wide association study of plasma efavirenz pharmacokinetics in AIDS Clinical Trials Group protocols implicates several CYP2B6 variants. *Pharmacogenet Genomics.* 2012;22(12):858-867.
17. Bertrand J, Chou M, Richardson DM, et al. Multiple genetic variants predict steady-state nevirapine clearance in HIV-infected Cambodians. *Pharmacogenetics and genomics.* 2012;22(12):868-876.

18. Kearns GL, Abdel-Rahman SM, Alander SW, Blowey DL, Leeder JS, Kauffman RE. Developmental pharmacology--drug disposition, action, and therapy in infants and children. *The New England journal of medicine.* 2003;349(12):1157-1167.

19. Ren Y, Nuttall JJ, Egbers C, et al. High prevalence of subtherapeutic plasma concentrations of efavirenz in children. *Journal of acquired immune deficiency syndromes.* 2007;45(2):133-136.

20. Pereira SA, Branco T, Corte-Real RM, et al. Long-term and concentration-dependent beneficial effect of efavirenz on HDL-cholesterol in HIV-infected patients. *Br J Clin Pharmacol.* 2006;61(5):601-604.

21. Autar RS, Boyd MA, Wit FW, et al. Relationships between drug exposure, changes in metabolic parameters and body fat in HIV-infected patients switched to a nucleoside sparing regimen. *Antivir Ther.* 2007;12(8):1265-1271.

22. Clevenbergh P, Garraffo R, Dellamonica P. Impact of various antiretroviral drugs and their plasma concentrations on plasma lipids in heavily pretreated HIV-infected patients. *HIV Clin Trials.* 2003;4(5):330-336.

23. WHO. Consolidated guidelines on the use of antiretroviral drugs for treating and prenting HIV infection. 2013; http://www.who.int/hiv/pub/guidelines/arv2013/download/en/.

24. Kampira E, Kumwenda J, van Oosterhout JJ, Dandara C. Mitochondrial DNA subhaplogroups L0a2 and L2a modify susceptibility to peripheral neuropathy in malawian adults on stavudine containing highly active antiretroviral therapy. *J Acquir Immune Defic Syndr.* 2013;63(5):647-652.

25. Hartkoorn RC, Kwan WS, Shallcross V, et al. HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by SLCO1B1 polymorphisms. *Pharmacogenet Genomics.* 2010;20(2):112-120.

26. Tebas P, Sension M, Arribas J, et al. Lipid levels and changes in body fat distribution in treatment-naive, HIV-1-Infected adults treated with rilpivirine or Efavirenz for 96 weeks in the ECHO and THRIVE trials. *Clin Infect Dis.* 2014;59(3):425-434.

27. Quercia R, Roberts J, Martin-Carpenter L, Zala C. Comparative changes of lipid levels in treatment-naive, HIV-1-infected adults treated with dolutegravir vs. efavirenz, raltegravir, and ritonavir-boosted darunavir-based regimens over 48 weeks. *Clin Drug Investig.* 2015;35(3):211-219.